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Evaluation of Dicamba Off-Target Movement and Subsequent Effects on Soybean Offspring

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Evaluation of Dicamba Off-Target Movement and Subsequent Effects on Soybean Offspring

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ABSTRACT

Commercial launch of cotton with resistance to dicamba, glyphosate, and glufosinate occurred in 2015 and launch of soybean with resistance to dicamba and glyphosate occurred in early 2016. It is likely that non-dicamba-resistant soybean will be planted in close proximity to dicamba-resistant soybean and cotton. Therefore, experiments were conducted to examine the distance dicamba moves during an application using commercial application equipment, as well as the effect the drift events have upon soybean offspring. Additional experiments were designed to investigate the effect glyphosate addition to dicamba has upon soybean growth and yield as well as possible effects on offspring. Lastly, an experiment was designed to determine the extent of secondary (volatile) drift of two formulations of dicamba under mid-summer conditions. Drift of dicamba exceeded 150 m in some drift trials (5% soybean injury). Drift trials established at early reproductive stages were more damaging to parent soybean; however, applications to late reproductive soybean were more detrimental to the soybean offspring. Percent of parent pods malformed resulting from dicamba drift events at the R5 growth stage displayed the highest correlation coefficients with offspring emergence ($r = -0.37$, $p = 0.0082$), vigor ($r = -0.57$, $p = < 0.0001$), injury ($r = 0.93$, $p = < 0.0001$), and amount of plants injured ($r = 0.92$, $p = < 0.0001$). When low rates of glyphosate were added to low rates of dicamba and applied to soybean at R1 growth stage, leaf malformation at 28 days after application (DAA) was increased over low rates of dicamba alone. Dicamba also caused damaging effects to soybean offspring; however, the addition of glyphosate did not increase further impact on soybean offspring. Diglycolamine (DGA) and N,N-Bis-(aminopropyl) methylamine (BAPMA) forms of dicamba are suspected to be similar in terms of primary drift; however, injury caused by secondary drift from BAPMA dicamba was less than DGA dicamba at 21 days after application (DAA). These results

document that caution should be used to minimize the risk for damage to neighboring non-dicamba-resistant soybean fields.

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

Chapter 1

Chapter 1 Appendices

Chapter 3

Chapter 4

General Introduction

Dicamba-resistant (DR) cotton (*Gossypium hirsutum* L.) and soybean (*Glycine max* L.) have been deregulated and were commercially launched in 2015 and 2016, respectively. However, approval by the Environmental Protection Agency (EPA) of a dicamba-containing herbicide for over-the-top applications to these crops was not given until November 9, 2016. Therefore, growers in some states were able to incorporate dicamba in the form of Xtendimax (Monsanto Corporation, St. Louis, MO 63167) or Engenia (BASF Corporation, Research Triangle Park, NC 27709) into dicamba-resistant (DR) crops in 2017.

Dicamba is a synthetic auxin herbicide in the benzoic acid family. Currently only six weeds have evolved resistance to dicamba worldwide (Heap 2017). However, only two incidences have occurred in the U.S. Kochia (*Kochia scoparia* L. Schrad.) has acquired resistance in populations in Kansas, Nebraska, Montana, North Dakota, Idaho, and Colorado. Populations in Idaho of prickly lettuce (*Latuca serriola* L. Lacse) have also conferred resistance to dicamba. The lack of evolved resistance may be good news to growers; however, history has demonstrated that increased herbicide reliance often quickly leads to resistance (Culpepper et al. 2006; Harker et al. 2017).

Much of the dicamba labeled for use before supplemental registration for DR cotton and soybean was applied in early spring as a preplant burndown application or in early vegetative applications to corn (*Zea mays* L.) or grain sorghum (*Sorghum bicolor* L. Moench.) prior to most soybean emergence (Anonymous 2014). Now that dicamba is labeled for in-crop use for DR cultivars, applications will be made much later in the growing season than current use patterns as applications will be allowed to soybean through R1 growth stage (Anonymous 2016a;

Anonymous 2016b). Therefore, the likelihood of applications being made when non-DR soybean have emerged in the nearby vicinity is great.

Approximately 50% of the agronomic crop hectares in Arkansas is annually planted to soybean (USDA 2016). Most growers may not want to plant a single variety, but rather choose to guard against economic loss by planting multiple varieties. Consequently, those choosing to plant DR soybean along with non-DR soybean will need to be aware of proper dicamba cleanout techniques to guard against damaging non-DR soybean in subsequent applications. Researchers have suggested that a single cleanout of spray tanks with an ammonia solution is not adequate to eliminate dicamba residue. Yet, two flushes with ammonia proved to be sufficient in removal of dicamba residue (Boerboom 2004). A triple-rinse procedure is commonly recommended to guard against sprayer contamination and subsequent exposure to a susceptible crop.

Exposure to non-DR soybean from primary (physical) drift of dicamba will not be reduced by new formulations. Applicators will need to be wary of environmental conditions during and soon after application of dicamba-containing products. Wind speeds have a near linear relationship with spray particle drift from ground applications (Maybank et al. 1978) and temperature inversions may result in off-target movement due to the inability of spray particles to settle to the soil surface. Furthermore, the type of spray equipment and how it is used will influence the risk for off-target movement via primary drift. Improper nozzle selection, application speed, and boom height can vastly increase the amount of primary spray drift that occurs (Maybank et al. 1978; Wolf et al. 1992).

Even after spray particles reach their intended site, subsequent volatilization may occur. Relative humidity and temperature are the primary factors affecting volatility (Egan and Mortenson 2012; Mueller et al. 2013). Increased temperature likewise causes greater risk for volatilization of dicamba. Furthermore, when low humidity accompanies high temperatures, risk for volatility further increases because there is greater available space in the atmosphere for dicamba to volatilize (Mueller et al. 2013). Volatilization of dicamba is possible from time of spraying up to three days after application, albeit most volatilization occurs in the first 12 hours after application (Behrens and Lueschen 1979; Mueller et al. 2013, Egan and Mortenson 2012). Nevertheless, studies have documented that as little as 1 mm of simulated rainfall will eliminate subsequent volatility (Behrens and Lueschen 1979).

Dicamba formulations have been known to differ in terms of likelihood and amount of volatility (Behrens and Lueschen 1979; Egan and Mortenson 2012; Mueller et al. 2013). The diglycolamine (DGA) salt of dicamba has been documented to have reduced secondary loss by 94% when compared to the dimethylamine (DMA) form of dicamba (Egan and Mortenson 2012). In other research, the sodium salt of dicamba was also found less volatile than the DMA form of dicamba (Behrens and Lueschen 1979).

If primary drift, secondary drift, or tank contamination occur, non-DR soybean will likely show symptoms within the first day to week after the event, depending on the dose incurred and rate of vegetative growth. Symptomology is commonly seen as chlorosis of terminal buds, cupping or crinkling of canopy leaves, swollen petiole bases, and leaf or stem epinasty (Auch and Arnold 1978; Sciumbato et al. 2004; Wax et al. 1969; Wiedenhamer et al. 1989). When soybean is exposed to higher drift rates of dicamba, stem cracking, terminal death, or plant termination may result (Griffin et al. 2013; Robinson et al. 2013; Solomon and Bradley 2014; Thompson and Egli 1973).

Growth stage at the time of the drift or contamination event will also play a role in injury to soybean. Dicamba exposure to soybean during vegetative stages does not always result in yield reduction (Al-Khatib and Peterson 1999; Andersen et al. 2004; Auch and Arnold 1978; Johnson et al. 2012; Kelley et al. 2005; Wax et al. 1969). However, early flowering stages are the most sensitive to yield reduction for non-DR soybean (Auch and Arnold 1978; Solomon and Bradley 2014; Wax et al. 1969).). Height reduction was also documented to accompany yield loss; yet, height reduction is not always an indicator of yield loss (Auch and Arnold 1978; Weidenhamer et al. 1989).

It is most likely that dicamba will be applied as a mixture or commercial premix with glyphosate in DR cotton and soybean to achieve broad-spectrum weed control. The addition of a full rate of glyphosate to low rates of dicamba can increase injury over the low rate of dicamba alone, when applied to glyphosate-resistant (GR) soybean (Kelley et al. 2005). Other research has documented an increase in control of glyphosate-resistant weeds by dicamba and glyphosate combinations over dicamba alone (Spaunhorst and Bradley 2013). As glufosinate-resistant soybean and conventional soybean acreage in Arkansas continues to increase in recent years, there may be increased risk for off-target movement and injury to soybean when dicamba is mixed with glyphosate over that of dicamba alone.

Information in the literature on the effect of dicamba on resulting offspring is limited; yet, deleterious effects of dicamba on offspring have been documented. Dicamba applied at 220 or 560 g ae ha^{-1} during flowering and podfill did not allow production of viable seed. Offspring from plants treated with dicamba at 11 to 56 g ha^{-1} at flowering and podfill reduced emergence from that of the nontreated check (Auch and Arnold 1978; Thompson and Egli 1973). Reductions in vigor were noticed in offspring from parents treated with dicamba at 30 g ha^{-1} (Thompson and Egli 1973). All seedling offspring displayed dicamba-like injury symptoms by the first trifoliate stage, with the most severe symptoms occurring for podfill applications. This is likely because the filling pod served as a strong sink for dicamba, which is mobile in the phloem of the plant (Senseman 2007). In addition, dicamba applied at flowering would have more time to be metabolized before podfill begins. Dicamba-like symptoms on offspring were transient and no injury was observed by the V2 stage of the offspring (Thompson and Egli 1973). There is no research in the literature pertaining to the effect of dicamba plus glyphosate combinations on soybean offspring; however, no negative effects to offspring were observed when glyphosate was applied from 8 to 420 g ae ha^{-1} at vegetative and reproductive stages (Norsworthy 2004).

Dicamba will be a useful tool for aiding in control of many glyphosate-resistant weeds and others that are difficult to control in current soybean production systems; however, precautions must be taken to reduce the possibility of off-target movement. New forms of dicamba may display a lower likelihood of volatility when compared to some previous forms, but off-target movement could still occur with poor stewardship, misapplication, or less than ideal environmental conditions at the time of application. In most cases, injury resulting from low rates of dicamba at vegetative stages should not reduce yield if terminal growth is not suspended (Auch and Arnold 1978; Weidenhamer et al. 1989). However, dicamba drift events during soybean reproductive development will likely cause greater risks for yield loss, and effects may be seen on the offspring in the form of reduced emergence and vigor (Thompson and Egli 1973). Further research is needed to understand the risks for off-target movement of dicamba, which should aid in establishing buffers to nearby sensitive crops. Effects of an actual drift event on soybean offspring are unknown; hence, they need to be investigated. Additionally, research quantifying possible differences in secondary drift of the DGA and BAPMA forms of dicamba is crucial before products are to be accepted for registration. Therefore, our objectives were: (1) to quantify the distance of off-target movement of the DGA form of dicamba to non-

DR soybean when applied using sprayer setup recommendations designed to minimize physical drift (Anonymous 2013), (2) to establish the relationship between direct damage to soybean plants with the appearance of dicamba-like symptomology or damage to soybean offspring following an actual drift event on the parent plants, (3) to assess damage to non-DR and non-glyphosate-resistant soybean when low rates of dicamba and glyphosate are applied during reproductive development as a mixture versus applying the herbicides alone, and (4) to determine the amount secondary drift occurring from two dicamba formulations under conditions that are likely during mid-summer applications in the Midsouth.

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Chapter 1

Off-Target Movement of Diglycolamine Dicamba to Non-Dicamba Soybean Using Practices to Minimize Primary Drift

Abstract

Soybean with resistance to dicamba (DR soybean) and glyphosate as well as cotton with resistance to glyphosate, glufosinate, and dicamba were recently commercialized in the US and have been readily adopted. To evaluate possible results of over-the-top application of dicamba in DR crops, field studies were designed to examine off-target movement using proposed sprayer setup recommendations. Association analysis and non-linear regression techniques were used to examine the effects of 26 field-scale drift trials conducted in 2014 and 2015 during soybean reproductive development (R1 through R6). The greatest predictors (injury, height reduction) of soybean yield reduction generally occurred and had steeper relationships after drift events at R1 growth stage than at later stages. Using non-DR soybean as an indicator, dicamba was documented to move as much as 152 m from the application area (distance to 5% injury). Instances of height reduction (5%) differed among growth stages with the greatest distance occurring at R1 (83.4 m). Soybean yield reduction was erratic with the greatest distance to 5% loss in yield occurring at 90.4 m after an R2 drift event. Overall, flowering stage soybean seems to be more sensitive than later reproductive soybean to injury, height reductions, and yield loss. Average and maximum wind speeds did not account for the injury documented, and it is hypothesized that other meteorological variables also play a notable role in dicamba off-target movement. With concerns of off-target movement of dicamba being on the forefront, proper stewardship of this new technology will be key to its longevity.

Nomenclature: dicamba; cotton, *Gossypium hirsutum* L.; soybean, *Glycine max* (L.) Merr.

Key words: Off-target movement, auxin-like symptomology, leaf malformation, pod malformation, soybean growth stages, dicamba volatility

Introduction

With resistance becoming more of a problem in broadleaf weeds such as Palmer amaranth (*Amaranthus palmeri* S. Wats.), waterhemp (*Amaranthus* spp.), and horseweed (*Conyza canadensis* L. Cronq.), there is a need for new herbicides to provide a different approach to control these broadleaf weeds in soybean and cotton (Kruger et al. 2010; Meyer et al. 2015). Research has documented that dicamba will effectively control these problem weeds and others when used properly in DR cotton and soybean (Byker et al. 2013; Cahoon et al. 2015). However, off-target movement to susceptible crops is of concern.

Off-target movement of pesticides can be complex in that spraying equipment, wind speed, crop stage, crop sensitivity, atmospheric conditions, and properties of the spray solution may all interact to influence the extent of a drift event (Heidary et al. 2014; Lofstrom et al. 2013). Many regulations have been put in place pertaining to these variables for dicamba application in DR soybean and cotton to limit off-target movement of the herbicide (Anonymous 2017a; Anonymous 2017b). However, lack of applicator training could still result in misapplications (Bish and Bradley 2017).

Off-target movement may occur as primary or secondary movement. Primary movement occurs at the time of application with the physical movement of spray droplets or evaporated particles from the target to an off-target site where a susceptible species may be growing. Influences on primary drift include droplet spectrum, wind speed, boom height, temperature, relative humidity, and spray pressure (Bueno et al. 2017; Maybank et al. 1974; Maybank et al. 1978; Threadgill and Smith 1975).

All herbicides have the potential for primary off-target movement; however, a select few also are subject to volatilization after application occurs. Previous dicamba products, such as the dimethylamine (DMA) salt formulation, have been known to readily cause volatile injury to nearby soybean (Behrens and Lueschen 1979). Increased temperature from 20 to 30 C is documented to double soybean response from volatility of the DMA salt of dicamba within closed chambers. Furthermore, reduced humidity was shown to also increase volatility in closed chamber experiments (Behrens and Lueschen 1979).

Recently, new lower volatile formulations of dicamba have been labeled for use in DR-crops (Anonymous 2017c; Anonymous 2017d). Xtendimax with VaporGrip (Monsanto Company, St. Louis, MO) is a combination of the previously available diglycolamine (DGA) form of dicamba and an additive that is claimed to reduce volatile losses by inhibition of free dicamba acid formation (MacInnes 2017). Additionally, the N,N-Bis-(aminopropyl) methylamine (BAPMA) form of dicamba (Engenia, BASF Corporation, Research Triangle Park, NC) was granted supplemental registration soon after. This form of dicamba is also purported to have reduced volatility over previous forms (Westberg and Adams 2017).

When supplemental labeling of Xtendimax with VaporGrip and Engenia occurred, only one nozzle was listed for use in DR soybean and cotton. Currently, 26 nozzles are approved for use in the application of Xtendimax with VaporGrip (Anonymous 2017a), whereas only 13 nozzles are allowed for use with Engenia (Anonymous 2017b). Nozzle selection is very important in achieving the desired droplet size to limit primary off-target movement (Heidary et al. 2014). Herbicides added to dicamba may also influence droplet size of the spray solution as the addition of *S*-metolachlor to Engenia was documented to reduce median droplet size by 28% when Turbo Teejet Air Inducted (TTI) nozzles were used (Meyer et al. 2016).

An ecological risk assessment for dicamba under the Endangered Species Act, using soybean as a bioindicator of risk based solely on plant height and weight reduction but not the presence of symptoms or yield loss, was completed by the Environmental Protection Agency (EPA) before approval of the herbicide for use in DR soybean and cotton (Anonymous 2017e; Environmental Protection Agency 2016). Subsequently, the Xtendimax with VaporGrip and Engenia labels both require a 33.3 m downwind application buffer to the field edge if vegetation exists such as a lawn or treeline due to the Endangered Species Act (Anonymous 2017c; 2017d). Hence, this buffer must be present from the last row treated to any non-crop vegetated area. However, buffers are not applicable when DR cotton or soybean are bordered by at least 33.3 m of DR cotton or soybean, corn (*Zea mays* L.), sorghum (*Sorghum bicolor* (L.) Moench), proso millet (*Panicum miliaceum* L.), small grains, sugarcane (*Saccharum officinale* L.), fields prepared for planting, areas covered by the footprint of a manmade structure with walls and roof, roads, paved surfaces, or graveled surfaces. Yet, under the language of the label, non-DR soybean may exist downwind of applications made to DR cotton and soybean if 33.3 m of the above-mentioned crops or structures are established in between. Though legal, the decision to spray in these circumstances is up to the applicator's discretion and is entirely their responsibility if damage to susceptible crops occur.

Soybean is highly sensitive to dicamba and may show visual injury such as leaf cupping at very low rate exposure (Auch and Arnold 1978; Weidenhamer et al. 1989). However, visual injury to soybean from dicamba does not always translate into yield reduction (Al-Khatib and Peterson 1999; Barber et al. 2017; Kelley et al. 2005; Solomon and Bradley 2014; Soltani et al. 2016; Weidenhamer et al. 1989; Westberg et al. 2016). Although some research has documented yield reduction to be similar among growth stages (Foster and Griffin 2016; Kelley et al. 2005; Weidenhamer et al. 1989) others have documented the early flowering stages (R1-R2) to be most yield limiting when compared to vegetative stages (Griffin et al. 2013; Robinson et al. 2013;

Soltani et al. 2016; Solomon and Bradley 2014). Conditions such as drought and high temperatures around the time of exposure to dicamba have been shown to influence soybean yield (Al-Khatib and Peterson 1999; Anderson et al 2004; Auch and Arnold 1978; Kelley et al. 2005; Weidenhamer et al. 1989). Soybean growth habit has also been cited to influence response to low rates of dicamba (Auch and Arnold 1978; McCown et al. 2016a; Wax et al. 1969; Weidenhamer et al. 1989). Therefore, the variability in yield loss among growth stages could possibly be due to environmental conditions or growth habit of soybean used in the conflicting studies.

Research studies have been conducted concerning herbicide drift from ground applications (Bueno et al. 2017; Heidary et al. 2014; Lofstrom et al. 2013). However, these studies were conducted at close range and attempted to quantify drift by using materials to catch particles to later be analyzed by laboratory equipment. Furthermore, the use of materials to catch drifting particles may underestimate or be unable to quantify the amount of dicamba reaching further distances because it may evaporate or volatize prior to settling. Because of the high sensitivity of soybean to dicamba, the crop is an excellent bioindicator to measure off-target movement. The objectives of this research were to: 1) identify the distance moved by a foliar application of the DGA salt of dicamba to reproductive soybean from a high-clearance sprayer using soybean injury, height, and yield as a bioindicators, 2) evaluate the correlations between soybean injury, height, pod malformation, and yield when exposed to a DGA dicamba drift event, and 3) determine the relationship between soybean response variables and the distance from the area where DGA dicamba was applied.

Materials and Methods

Twenty-five field experiments were conducted in 2014 and 2015 at the Northeast Research and Extension Center in Keiser, Arkansas, to examine off-target movement of DGA dicamba using a sprayer setup that was anticipated as requirements for applying new formulations of dicamba in DR soybean and cotton (Anonymous 2013; Anonymous 2014). One additional experiment was conducted at the Lon Mann Cotton Research Station (LMCRS) near Marianna, Arkansas, in 2015. All drift experiments were conducted using the commercially available DGA formulation of dicamba branded Clarity® (BASF Corporation, Raleigh, NC). Timing for dicamba applications was restricted to the reproductive stages of R1 through R6. Dicamba was applied at 560 g ae ha^{-1} using a Bowman Mudmaster Sprayer (Bowman Manufacturing, Newport, AR 72112) traveling 16 km h^{-1}. The high-clearance sprayer was equipped with TeeJet AIXR 11003 nozzles (TeeJet Technologies, Wheaton, IL 60187) and calibrated to deliver 93.5 L ha^{-1} at 275 kPa to achieve a very-coarse droplet spectrum. It is acknowledged that the current nozzles recommended for the new formulations of dicamba do not include AIXR 11003 nozzles. Rather, the current labels permit use of certain nozzles that produce either an extremely-coarse or ultra-course droplet spectrum. However, at the beginning of this study it was publicized that very-coarse spray spectrums, along with outputs of 93.5 L ha^{-1} would be allowed for dicamba application (Anonymous 2013; Anonymous 2014).

The application area was 8 by 30 m in size where the wind blew parallel or less than 45 degrees to the soybean rows (Figure 1A) and 8 by 60 m in size where the wind blew perpendicular or greater than 45 degrees to the soybean rows (Figure 1B). Handheld Kestrel anemometers (Nielson-Kellerman CO, Birmingham, MI) were used to record wind speed every second during applications. Angle of wind direction, temperature, and relative humidity were also recorded at the time of application. At 28 days after application (DAA) in experiments where the wind was greater than 45 degrees from the soybean rows, three transects were established across rows extending downwind from the area sprayed (Figure 1B). The centers of transects were initiated at 18, 30, and 42 m into the 60-m application swath. Each plot was four rows, spaced 96 cm and 12 m in length, with only the center two being used for data collection. Plots extended along transects until no injury was observed or the end of the field was reached. In experiments where the wind was less than 45 degrees from the soybean rows, transects were laid out extending downwind from the center and to the left and right side of the downwind edge of the 8- by 30-m application area in four-row increments until no injury was observed laterally. Plots were established down rows in 6-m lengths until no injury was observed. Again, rows were spaced 96 cm, and data were collected from the center two of four rows. Grid coordinates were given to each plot with x=0 and y=0 being the center of the downwind edge of the application.

Soybean injury and three canopy heights were recorded at 28 DAA for each plot. A visual scale from 0 to 100%, with 100% being plant death, was used to estimate soybean injury. The percent of pods malformed and the height to the terminal of three individual plants per plot were recorded at soybean maturity. Both canopy height and mature height were converted to a percent relative to uninjured plots by selecting three random plots having 0% soybean injury (outside of the drift plume) at 28 DAA. Percentage of pods malformed were recorded on a 0 to 100% scale, with 0 being no pod malformation and 100 being all pods having malformation. A small-plot combine was used to harvest plots, and grain yields were corrected to 13% moisture before being converted to a percentage yield relative to uninjured plots.

Correlation analysis was conducted using JMP Pro 12 (SAS Institute, Cary, NC) and Pearson pairwise correlations were produced between injury at 28 DAA, relative canopy height at 28 DAA, percentage of mature pods malformed, relative terminal height at maturity, and relative yield. Contour maps were constructed using SAS 9.4 (SAS Institute, Cary, NC) for each drift trial illustrating 28 DAA injury, percent of pods malformed at maturity, mature relative terminal height, and relative yield. Regression analysis was performed using a single line of data closest to the center of the drift plume as determined by the contour maps in conjunction with injury ratings and exact distance to the center of each plot from the center of the edge of the application area. Essentially, the plot reported to have the highest amount of injury at 28 DAA in each transect was closest to the center of the drift plume. These same plots were used in the regressions for 28 DAA relative canopy height, mature percent of pods malformed, mature relative terminal height, and relative yield. Because the location of each plot was represented by an x and y value, exact distance to the center of each plot was computed using the Pythagorean Theorem. These data were used to construct models to determine the distance to 5% injury at 28 DAA, 5% canopy height reduction at 28 DAA, 5% terminal height reduction at maturity, mature pod malformation of 5%, and 5% yield loss for each drift event. The regression models were tested using Sigma Plot (Systat Software Inc., San Jose, CA) regarding significance ($\alpha = 0.05$) and goodness of fit (r^2, AIC, BIC). Exponential models have been used previously to describe spray deposition as a function of distance from a drift event (Bueno et al. 2017). Therefore, one, two, three, and four parameter models were tested to decide the best fit. Measures of AIC and BIC were used to compare across models with the lowest values indicating the best fit. Regression figures were assembled using JMP 13 Pro (SAS Institute, Cary, NC).

Results and Discussion

Correlation analysis between soybean injury, height, pod malformation, and yield. Generally, relationships between parameters evaluated were stronger following drift events at flowering stages (R1 to R2) than at pod (R3 to R4) or seed-forming stages (R5 to R6) (Tables 1, 2; Figures 2 through 7). Correlations between observations were greatest when the drift events occurred at R1 growth stage likely because the opportunity for growth prior to maturity is greater at R1 growth stage (Table 1). Previous research has also documented the flowering stages to be most sensitive to yield loss compared to vegetative or later reproductive stages (Auch and Arnold 1978; Griffin et al. 2013; Robinson et al. 2013; Solomon and Bradley 2014; Wax et al. 1969).

Soybean injury associated with R1 and R2 drift events was often two-fold the injury seen in later drift events (Figures 2 and 3). When soybean is exposed to dicamba, the effects are only seen in new growth because dicamba translocates to newly formed meristematic tissue (Senseman 2007). Therefore, injury seen after early reproductive soybean is exposed to dicamba will primarily be noticed as leaf cupping because vegetative growth is still occurring at a rapid pace in indeterminate cultivars (Heatherly and Elmore 2004). When pod formation begins (R3), vegetative growth slows considerably, resulting in less visible soybean injury (Figure 4).

Although not tested statistically, the impact on soybean height and pod malformation seems to differ across growth stages (Figures 2 through 7). Reductions in height seem to be more common at earlier reproductive stages. As soybean plants approach maturity, there is less capacity for height reduction because plants are at or near maximum height. The percentage of soybean pods malformed was as high as 80% for R2, 70% for R3, and 60% for R1 drift events (Figures 2 through 4). Because all varieties used in these studies were indeterminate in growth habit, pod malformation was still noticed in the upper nodes of soybean plants at up to 15% for R4 and R5 and 5% for R6 drift trials (Figures 5 through 7).

Correlation analysis between wind and distance moved. An additional correlation analysis was performed between wind speed data during application and calculated distance to 5% soybean injury (data not shown). Neither maximum nor average wind speed was significantly correlated with the distance to 5% soybean injury. Although wind speed has been documented to greatly affect drift of pesticides, atmospheric conditions such as thermals, temperature, and humidity could play a vital role in dicamba off-target movement. Other research also observed the amount of 2,4-D drift (also a synthetic auxin) not to be solely dependent on average wind speeds (Wolf et al. 1992).

Previous research has examined several meteorological parameters and their effect on particle drift (Threadgill and Smith 1975). A distinct observation of greater drift when unstable atmospheres occurred at the time of application was documented; thus, temperature gradients involving lower temperatures at the crop lead to upward movement of air (thermals) to warmer temperature above the surface. The updraft essentially allowed for particles to remain suspended for longer periods of time. Furthermore, increased wind speeds lead to decreased particle drift in some cases because, as the authors suggest, the atmosphere becomes homogenized when warm and cool air masses mix, basically eliminating updrafts.

When temperatures are high (32 C and above) evaporation of spray droplets may occur before they reach their intended site (Maybank et al. 1974). The more solution that evaporates from a spray particle, the lighter it will become and therefore may travel further before deposition. In the case of dicamba, evaporation of its carrier could in turn lead to volatility. Volatility after application could have occurred because of the impact of temperature and humidity. However, Maybank et al. (1974) did not record temperature and humidity following application to use for analysis. In the present study, considerable upwind injury or injury in multiple directions was noticed in some applications, likely attributable to volatile movement of dicamba.

In addition to the effect of temperature on spray particle movement, environmental conditions at the time application and soon after could affect the extent of symptomology and ability of soybean plants to recover from dicamba exposure. Previous research documented that dry conditions increased the sensitivity of soybean to dicamba (Andersen et al. 2004; Auch and Arnold 1978; Kelley et al. 2005; Weidenhammer et al. 1989). Furthermore, higher temperatures near the time of exposure resulted in increased sensitivity of soybean to dicamba (Al-Khatib and Peterson 1999).

Non-linear regression models. Three-parameter exponential models were a good fit for relating soybean variables with distance from the applied area (Appendix Figures 1 through 25). Drift trials occurring at R6 were not included in this analysis because injury symptoms were only observed near the application, which resulted in these trials only spanning twenty meters from the treated area.

Soybean injury at 28 DAA was adequately described using the model, with all R^2 being greater than 0.91 for all trials, regardless of growth stage (Appendix Figures 1 through 5). Yet, because of differences in soybean sensitivity to dicamba among growth stages, trials must be compared within each growth stage. As expected, the distance that dicamba injury to soybean could be visibly detected decreased after flowering stages (R1-R2) (Table 3). For R1 applications, a maximum distance of 128.2 m was documented (maximum wind 19 km h^{-1}, average wind 16.9 km h^{-1}), with distance increasing to 152 m at R2 when wind speeds were less (maximum wind 15.4 km h^{-1}, average wind 11.1 km h^{-1}).

Height reduction at 28 DAA and at maturity followed similar trends as injury in that less height reduction was seen as application was delayed. Previous research documents that mature height reduction occurs more at early reproductive stages than at later reproductive stages (Auch and Arnold 1978). The distance to 5% harvest height reduction was greater than canopy height reduction at 28 DAA after R1 drift events but was less than or equal to R1 drift at later drift applications. The fact that average height reduction decreased from 28 DAA to maturity after R1 drift events indicates that soybean nodes added later than 28 days after R1 growth stage may be affected by dicamba. However, this parameter was not investigated in this study.

This research documents that height reduction to non-DR soybean can occur at greater distances than those listed on the Xtendimax and Engenia labels when AIXR 11003 nozzles are used with an output of 93.5 L ha^{-1}. In some cases, 5% height reduction occurred at over 80 m, which is over twice the required buffer for endangered species when using an approved nozzle.

The average distance to 5% pod malformation was numerically greater after R2 drift events than R1 drift events, indicating soybean could be more sensitive to pod malformation from dicamba drift at this stage. Pod malformation may be an indicator that dicamba has been translocated to pods and/or seeds. Previous research documented pod malformation to occur after exposure to dicamba and for subsequent offspring to be malformed in some cases (Barber et al. 2015; McCown et al. 2016b; Thompson and Egli 1973). Furthermore, auxin symptomology occurrence in newly planted soybean could be blamed on drift exposure, which may cause dicamba complaints to be filed where they are unwarranted.

Two trials after R1 drift events (33.9 m, 42.8 m), two trials after R2 drift events (40.9 m, 90.4 m), and one trial after a R3 drift event (33.5 m) were documented to cause 5% yield loss to soybean beyond the buffer distance established for endangered species at the field edge. Using the sprayer setup evaluated in this research, dicamba application in DR cotton and soybean may lead to yield loss beyond a 33.3 m buffer in the downwind direction and the risk may increase relative to the size of the treated area. In this research, no more than 480 m^2 were treated, and it should be noted that only a single pass of a sprayer was utilized. There would be opportunity to increase primary drift exposure to downwind species if multiple passes were used.

Based on label guidelines, application would be permissible where non-DR soybean is bordering DR cotton or soybean, but the wind direction would have to be directly away from sensitive crops such as non-DR soybean at the time of application (Anonymous 2017c; Anonymous 2017d). Even so, volatility of DGA dicamba, including the new formulations, can occur at least 3 days after application (Jacobson et al. 2016a; Jacobson et al. 2016b; Mueller et al. 2013). Because of volatilization and other forms of possible secondary movement, it is not possible to conclude that all of the injury or damage observed in these trials was solely the result of primary drift. With injury sometimes observed in directions other than those that were downwind at application, future efforts should try to quantify soybean response to the separate contributions of primary and secondary off-target movement of dicamba.

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Table 1. Correlation coefficients for soybean injury, height at 28 DAA, height at harvest, pod malformation, and yield after a diglycolamine dicamba drift event.[a]

	GS[b]	Injury at 28 DAA[c]	Height at 28 DAA[d]	Height at harvest[d]	Pod malformation[e]	Yield[d]
Injury at 28 DAA[b]	R1		-0.7777*	-0.6790*	0.8477*	-0.5055*
	R2		-0.1691*	-0.3989*	0.7887*	NS
	R3		-0.6153*	-0.3927*	0.6557*	-0.2673*
	R4		NS	-0.2203	0.5806*	-0.4575*
	R5		NS	NS	0.8401*	0.4315*
	R6		-	-	-	-
Height at 28 DAA[c]	R1	-0.7777*		0.8219*	-0.8589*	0.6157*
	R2	-0.1691*		0.412*	-0.3833*	0.0965
	R3	-0.6153*		0.4641*	-0.4734*	0.297*
	R4	NS		NS	0.284*	NS
	R5	NS		NS	NS	0.3105
	R6	-		-	-	-
Height at harvest[c]	R1	-0.6790*	0.8219*		-0.8314*	0.6687*
	R2	-0.3989*	0.412*		-0.5986*	0.1389
	R3	-0.3927*	0.4641*		-0.2268*	0.314*
	R4	-0.2203	NS		NS	NS
	R5	NS	NS		NS	0.3788*
	R6	-	-		NS	0.4622*
Pod malformation[d]	R1	0.8477*	-0.8589*	-0.8314*		-0.6535*
	R2	0.7887*	-0.3833*	-0.5986*		NS
	R3	0.6557*	-0.4734*	-0.2268*		-0.1122
	R4	0.5806*	0.284*	NS		-0.2991
	R5	0.8401*	NS	NS		0.3593*

Table 1 continued

	GS[b]	Injury at 28 DAA[c]	Height at 28 DAA[d]	Height at harvest[d]	Pod malformation[e]	Yield[d]
	R6	-	-	NS		NS
Yield[c]	R1	-0.5055	0.6157	0.6687	-0.6535	
	R2	NS	0.0965	0.1389	NS	
	R3	-0.2673*	0.297*	0.314*	-0.1122	
	R4	-0.4575*	NS	NS	-0.2991	
	R5	0.4315*	0.3105	0.3788*	0.3593*	
	R6	-	-	0.4622*	NS	

Abbreviations: DAA = days after application; GS = growth stage; * = significance to $\alpha \leq 0.01$; NS = not significant

[a]Correlation coefficients were computed on a pairwise method

[b]Sample sizes: R1(481), R2(557), R3(333), R4(118), R5(81), R6(66)

[c]Soybean injury was rated on a 0 to 100% scale with 100% being plant death

[d]Heights and yield were converted to a percentage of the uninjured, with the uninjured being the average of 3 random plots within each trial having no injury at 28 DAA

[e]Pod malformation ratings were taken as a percentage of pods malformed per plant

Table 2. Correlation coefficient confidence intervals (95%) for soybean injury, height at 28 DAA, height at harvest, pod malformation, and yield.

		Injury at 28 DAA[a]		Height at 28 DAA[b]		Height at harvest[b]		Pod malformation[c]		Yield[b]	
	GS[d]	Lower	Upper	Lower	Upper	Lower	Upper	Lower	Upper	Lower	Upper
Injury at 28 DAA[a]	R1			-0.811	-0.7393	-0.7255	-0.6264	0.8205	0.8711	-0.5692	-0.4358
	R2			-0.2521	-0.0837	-0.4667	-0.3265	0.755	0.8182	-	-
	R3			-0.6787	-0.5427	-0.4814	-0.2959	0.5897	0.713	-0.3658	-0.1629
	R4			-	-	-0.3857	-0.0411	0.4467	0.689	-0.5969	-0.2914
	R5			-	-	-	-	0.7603	0.908	0.174	0.6337
	R6			-	-	-	-	-	-	-	-
Height at 28 DAA[b]	R1	-0.811	-0.7393			0.7894	0.8498	-0.8808	-0.8332	0.5564	0.6688
	R2	-0.2521	-0.0837			0.3372	0.4816	-0.455	-0.3067	0.0098	0.1828
	R3	-0.6787	-0.5427			0.3727	0.5466	-0.5538	-0.3842	0.1942	0.3934
	R4	-	-			-	-	0.1089	0.4421	-	-
	R5	-	-			-	-	-	-	0.0352	0.542
	R6	-	-			-	-	-	-	-	-
Height at harvest[b]	R1	-0.7255	-0.6264	0.7894	0.8498			-0.8577	-0.8008	0.6147	0.7164
	R2	-0.4667	-0.3265	0.3372	0.4816			-0.6495	-0.5424	0.0554	0.2204
	R3	-0.4814	-0.2959	0.3727	0.5466			-0.328	-0.1205	0.2106	0.4104
	R4	-0.3857	-0.0411	-	-			-	-	-	-
	R5	-	-	-	-			-	-	0.1596	0.5624
	R6	-	-	-	-			-	-	0.2138	0.6544
Pod malformation[c]	R1	0.8205	0.8711	-0.8808	-0.8332	-0.8577	-0.8008			-0.7019	-0.5991
	R2	0.755	0.8182	-0.455	-0.3067	-0.6495	-0.5424			-	-
	R3	0.5897	0.713	-0.5538	-0.3842	-0.328	-0.1205			-0.2186	-0.0031
	R4	0.4467	0.689	0.1089	0.4421	-	-			-0.4642	-0.114
	R5	0.7603	0.908	-	-	-	-			0.1375	0.5468

Table 2 continued

	GS[d]	Injury at 28 DAA[a]		Height at 28 DAA[b]		Height at harvest[b]		Pod malformation[c]		Yield[b]	
		Lower	Upper	Lower	Upper	Lower	Upper	Lower	Upper	Lower	Upper
	R6	-	-	-	-	-	-			-	-
Yield[b]	R1	-0.5692	-0.4358	0.5564	0.6688	0.6147	0.7164	-0.7019	-0.5991		
	R2	-	-	0.0098	0.1828	0.0554	0.2204	-	-		
	R3	-0.3658	-0.1629	0.1942	0.3934	0.2106	0.4104	-0.2186	-0.0031		
	R4	-0.5969	-0.2914	-	-	-	-	-0.4642	-0.114		
	R5	0.174	0.6337	0.0352	0.542	0.1596	0.5624	0.1375	0.5468		
	R6	-	-	-	-	0.2138	0.6544	-	-		

Abbreviations: DAA = days after application; GS = growth stage

[a]Soybean injury was rated on a 0 to 100% scale with 100% being plant death

[b]Heights and yield were converted to a percentage of the uninjured, with the uninjured being the average of 3 random plots within each trial having no injury at 28 DAA

[c]Pod malformation ratings were rated as a percentage of pods malformed per plant

[d]Sample sizes: R1(481), R2(557), R3(333), R4(118), R5(81), R6(66)

Table 3. Growth stage, and maximum and average wind speeds during application and the calculated distance to 5% observed soybean injury, 5% reduction in height at 28 days after application, 5% reduction in height at harvest, 5% pod malformation, and 5% reduction in yield for drift trials.[a,b]

		Wind speeds during application[c]						
Growth stage	Trial	Maximum	Average	Calculated distance to 5% soybean injury	Calculated distance to 5% height reduction at 28 DAA	Calculated distance to 5% height reduction at harvest	Calculated distance to 5% pod malformation at harvest	Calculated distance to 5% yield reduction
		------km h^{-1}------		----------m----------				
R1	1	19.0	16.9	128.2	49.6	72.8	85.6	25.9
	2	19.8	15.1	94.1	42.1	79.2	54.4	14.6
	3	19.3	16.3	91.6	38.5	75.1	66.3	33.9
	4	18.0	15.8	120.1	83.0	51.5	77.6	9.7
	5	16.8	12.1	75.1	52.1	24.0	41.4	18.5
	6	15.3	16.3	64.4	36.8	83.4	49.6	42.8
R2	7	14.5	12.6	36.4	53.3[d]	42.4	40.6	40.9
	8	17.7	14.9	85.5	34.0	36.6	52.4	10.1
	9	11.9	10.2	116.7	54.3	23.4	95.0	0[e]
	10	15.4	11.1	152.0	14.5	17.0	139.5	3.7
	11	12.9	12.1	60.6	0[e]	15.4	60.6	90.4[d]
	12	13.7	8.5	30.3	-[f]	0[e]	36.2	0[e]
R3	13	10.5	9.1	39.2	8.2	6.6	25.1	5.7
	14	15.3	14.0	30.0	0[e]	0[e]	27.9	11.2
	15	21.2	16.2	61.0[d]	36.2	7.5	34.1	0[e]
	16	14.6	12.6	50.3	24.1	22.1	23.4	33.5

Table 3 continued

Growth Stage	Trial	Wind speeds during application[c]		Calculated distance to 5% soybean injury	Calculated distance to 5% height reduction at 28 DAA	Calculated distance to 5% height reduction at harvest	Calculated distance to 5% pod malformation at harvest	Calculated distance to 5% reduction in yield
		Maximum	Average					
	17	14.3	11.2	16.5	0[e]	0[e]	18.1	0[e]
R4	18	15.6	13.1	17.0	0[e]	0[e]	22.7	10.2
	19	14.6	13.4	16.1	8.2	0[e]	2.8	0[e]
R5	20	14.6	13.4	27.0	0[e]	0[e]	15.7	0[e]

[a]Trials with less than 5 data points were excluded from the analysis
[b]Distances were calculated using the reverse prediction function in JMP Pro 13 (SAS Institute, Cary, NC)
[c]Wind speeds were recorded at 1 sec intervals during application.
[d]Value recorded from the equation resulted in extrapolation; therefore, the furthest distance where data were recorded is used.
[e]Not a significant regression; therefore, a distance of 0 m was used.
[f]Data not recorded.

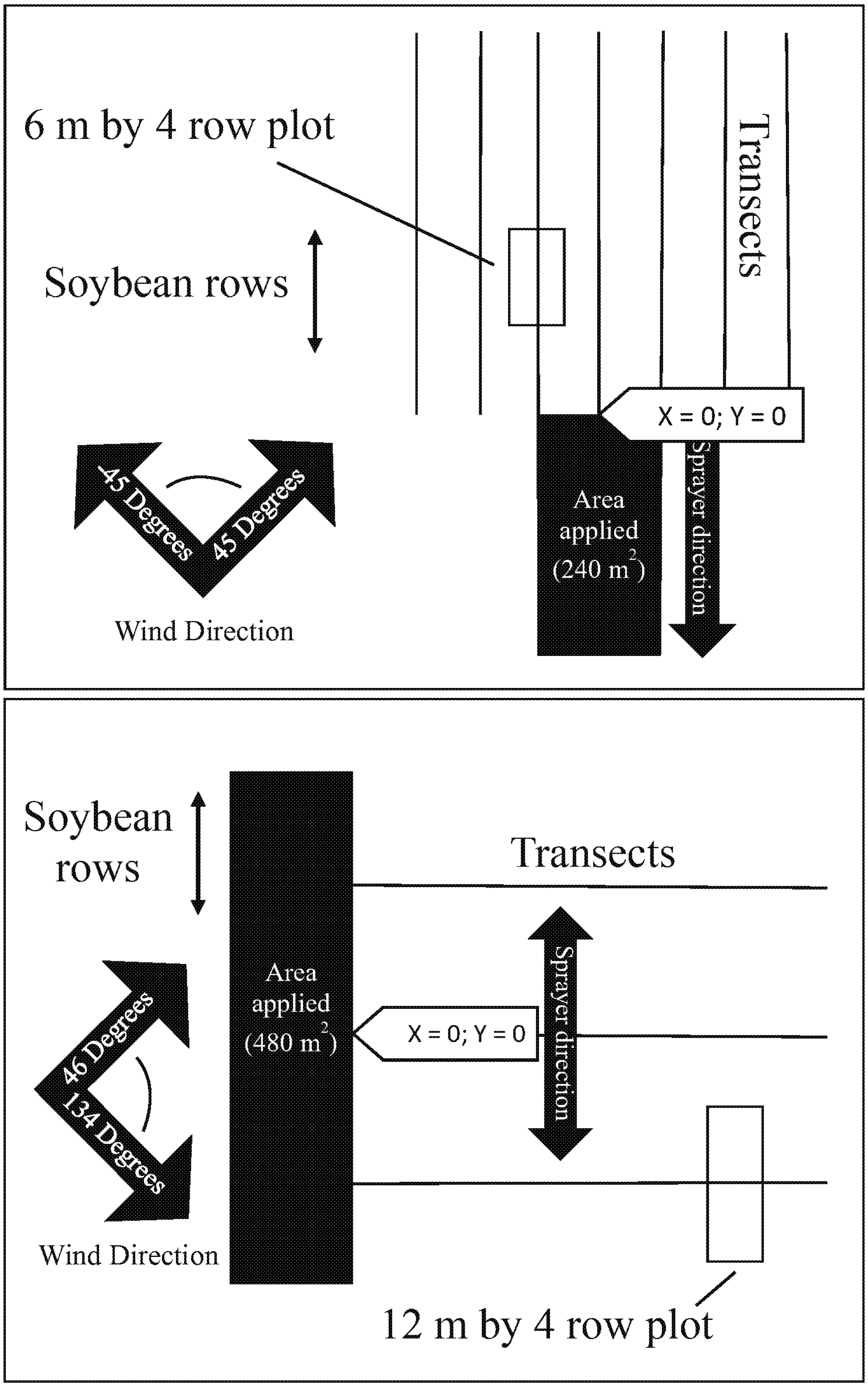


Figure 1. Design of drift trials with wind predominately occurring (A) down rows and (B) across rows.

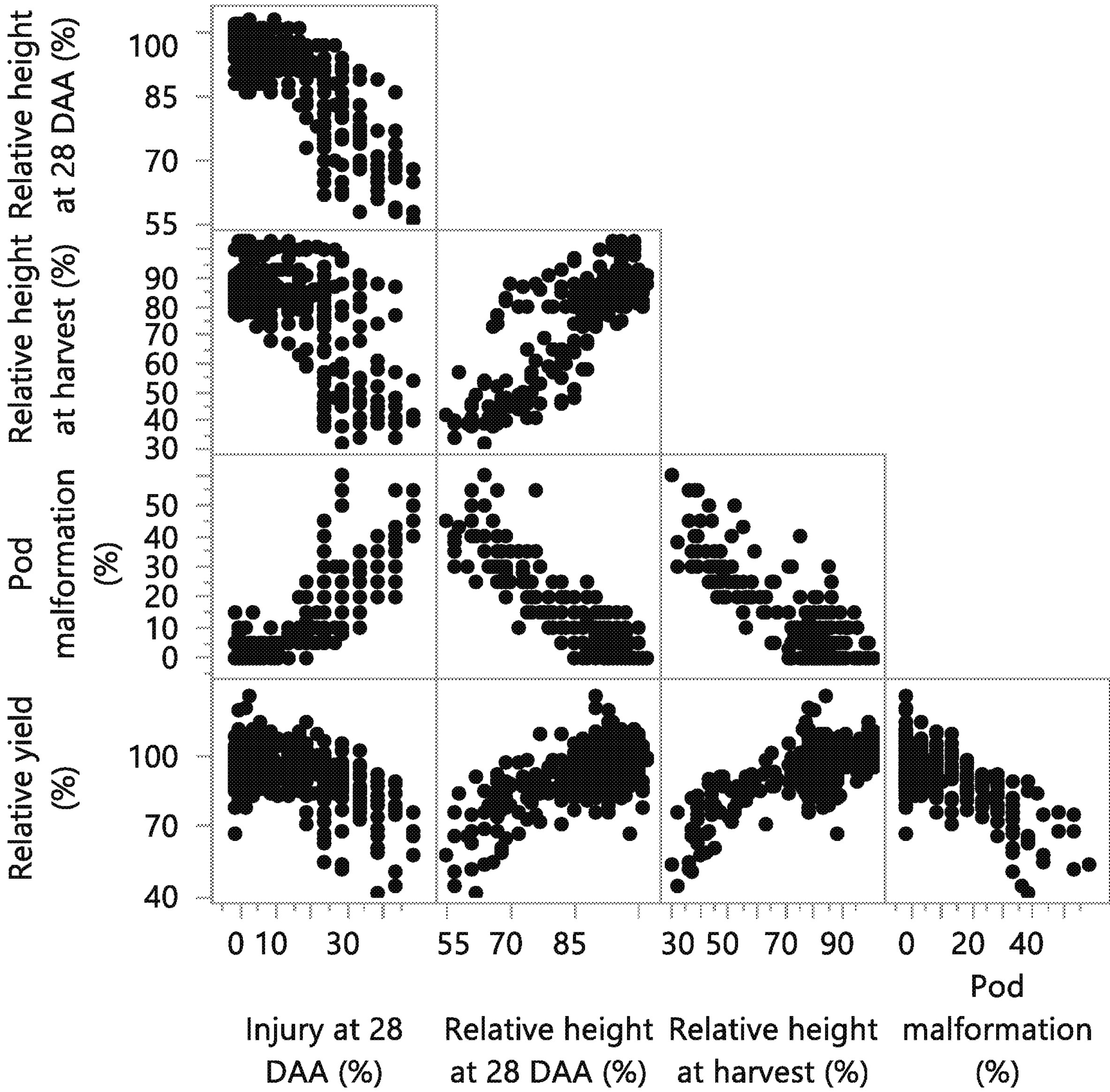


Figure 2. Scatterplot matrix of soybean observations after a diglycolamine dicamba drift event at R1. Heights and yield are reported as percentage of the uninjured. Uninjured is referring to the average of three random plots outside of the drift plume that were recorded to have no visual injury at 28 DAA.

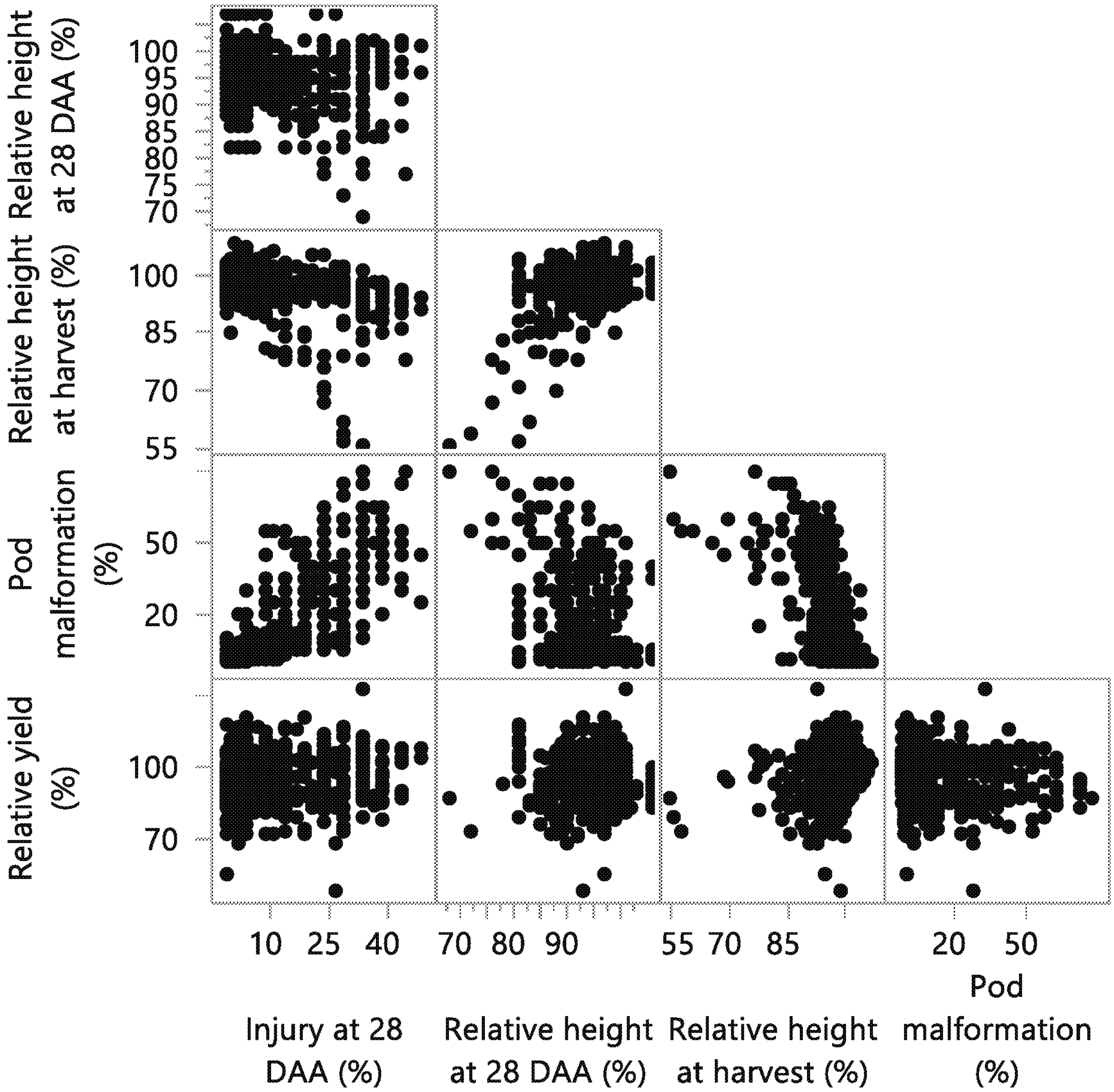


Figure 3. Scatterplot matrix of soybean observations after a diglycolamine dicamba drift event at R2. Heights and yield are reported as percentage of the uninjured. Uninjured is referring to the average of three random plots outside of the drift plume that were recorded to have no visual injury at 28 DAA.

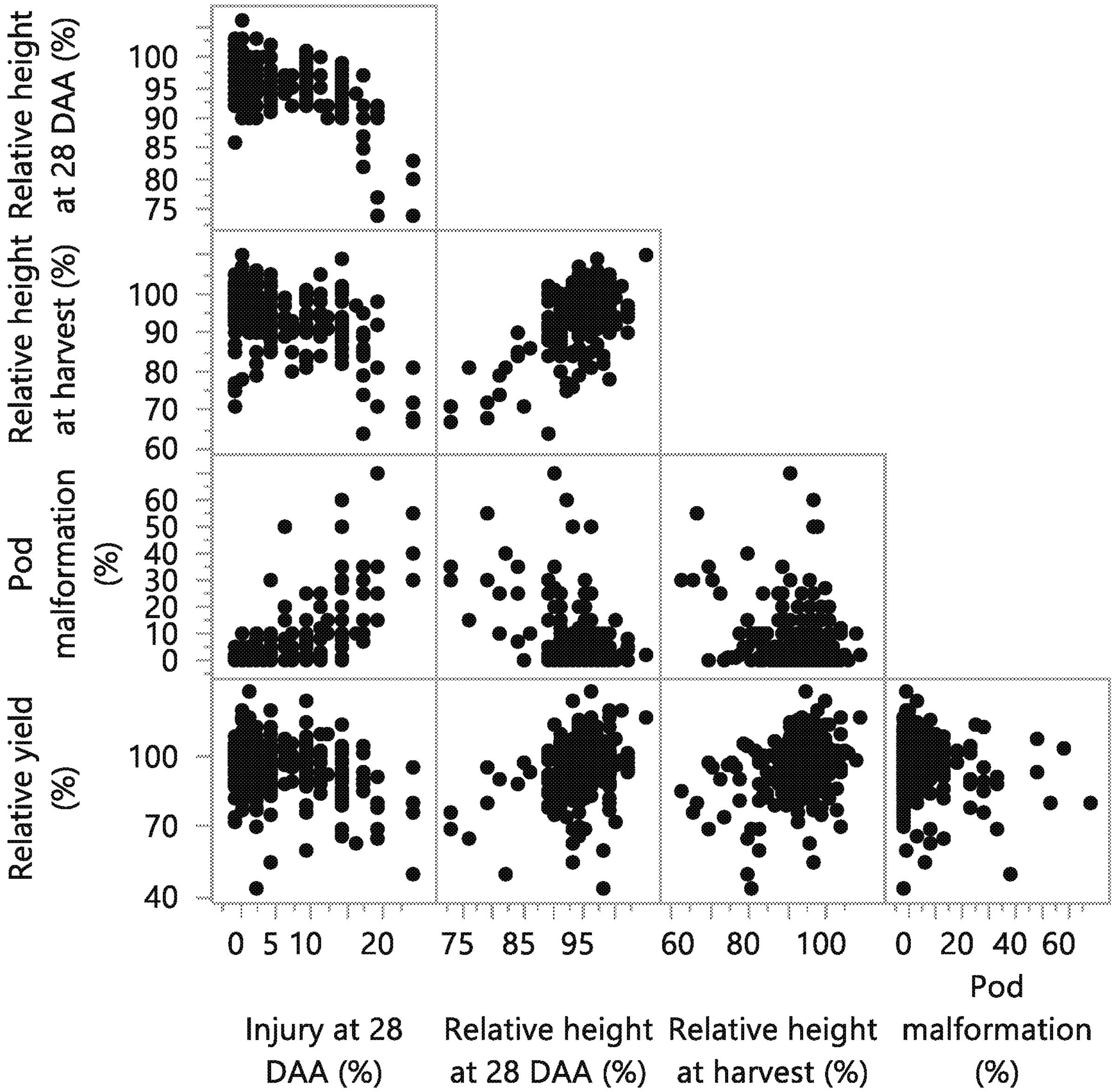


Figure 4. Scatterplot matrix of soybean observations after a diglycolamine dicamba drift event at R3. Heights and yield are reported as percentage of the uninjured. Uninjured is referring to the average of three random plots outside of the drift plume that were recorded to have no visual injury at 28 DAA.

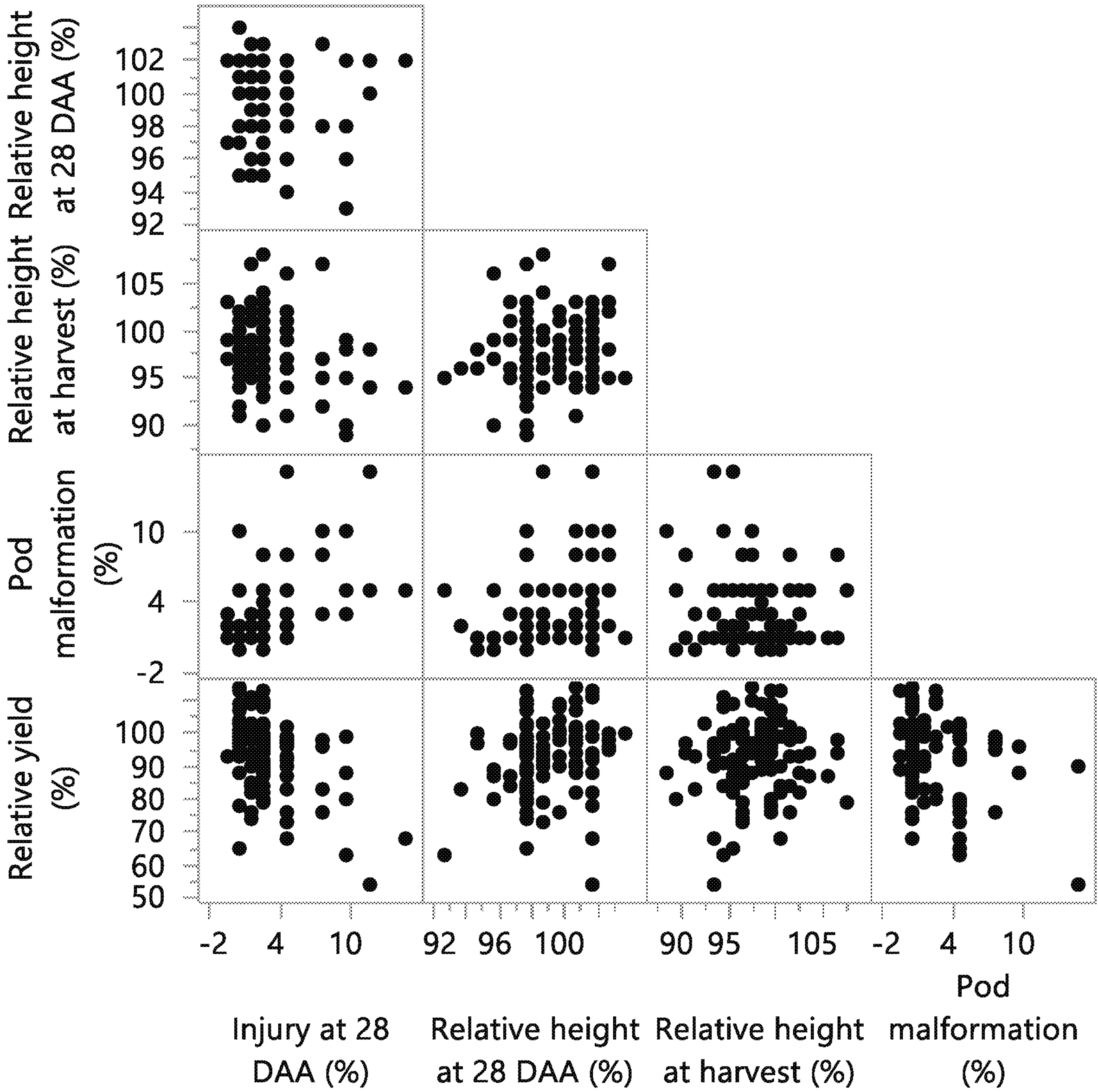


Figure 5. Scatterplot matrix of soybean observations after a diglycolamine dicamba drift event at R4. Heights and yield are reported as percentage of the uninjured. Uninjured is referring to the average of three random plots outside of the drift plume that were recorded to have no visual injury at 28 DAA.

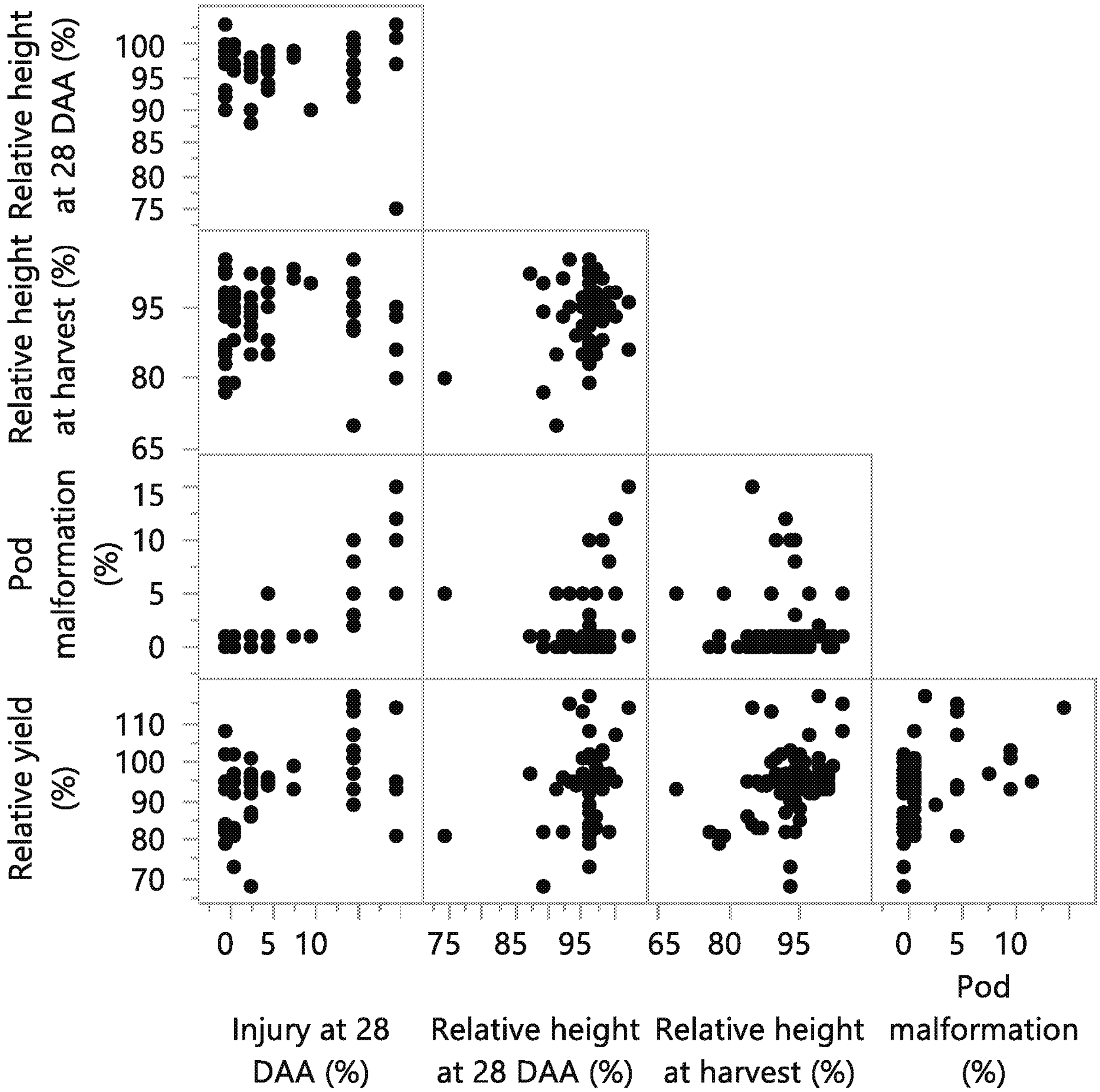


Figure 6. Scatterplot matrix of soybean observations after a diglycolamine dicamba drift event at R5. Heights and yield are reported as percentage of the uninjured. Uninjured is referring to the average of three random plots outside of the drift plume that were recorded to have no visual injury at 28 DAA.

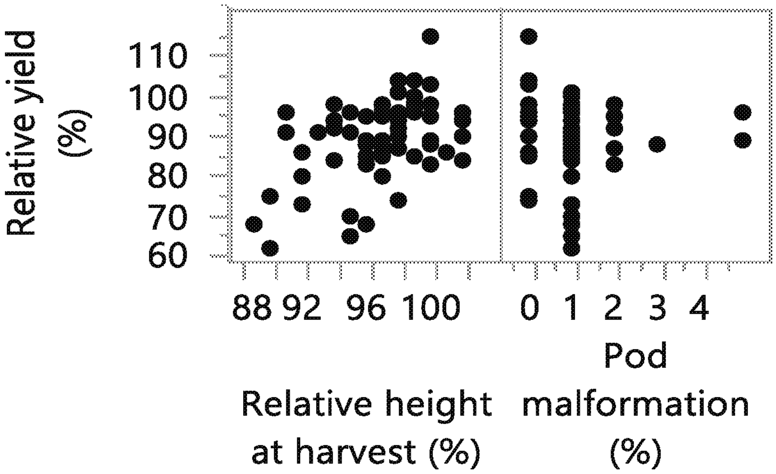
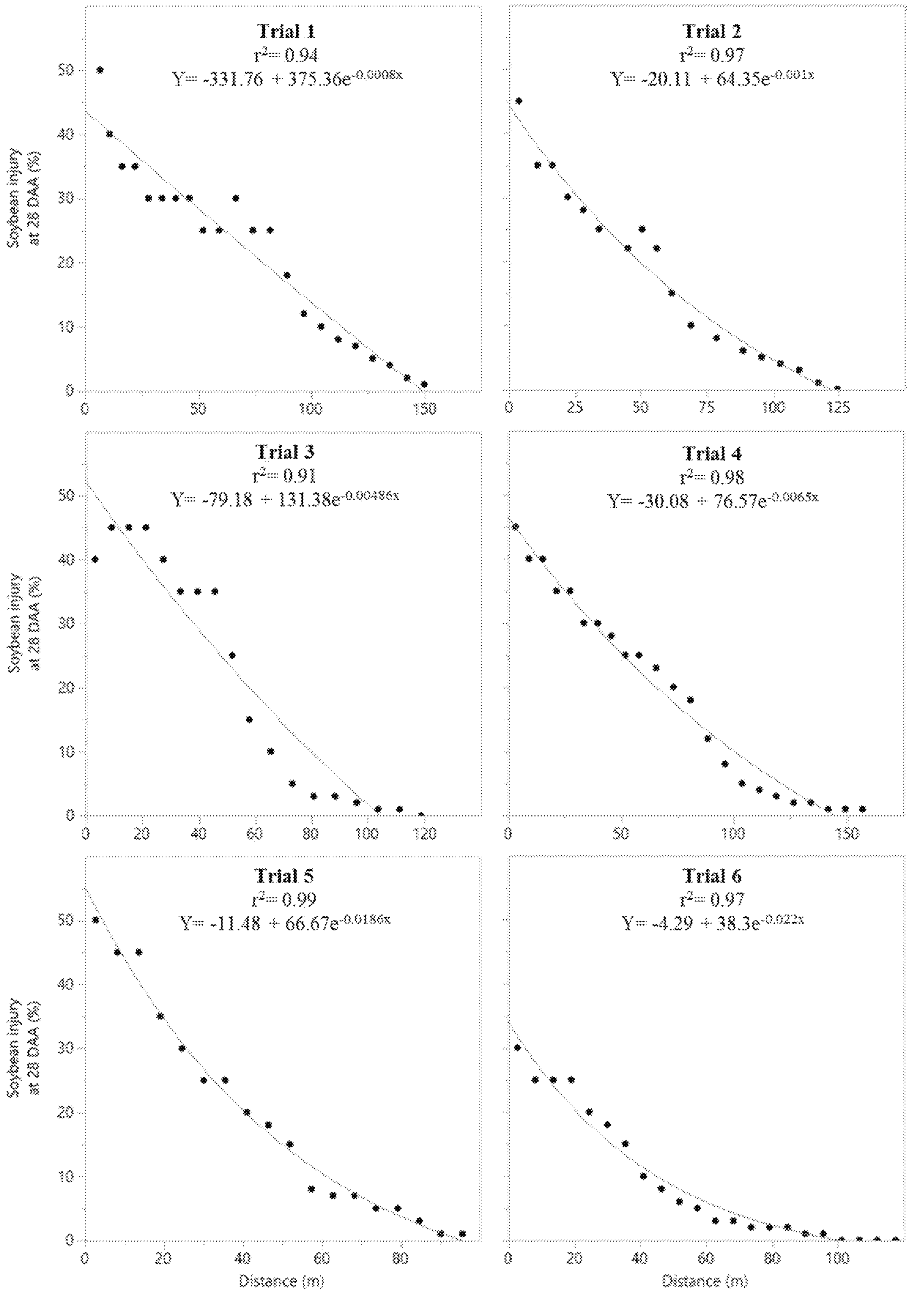
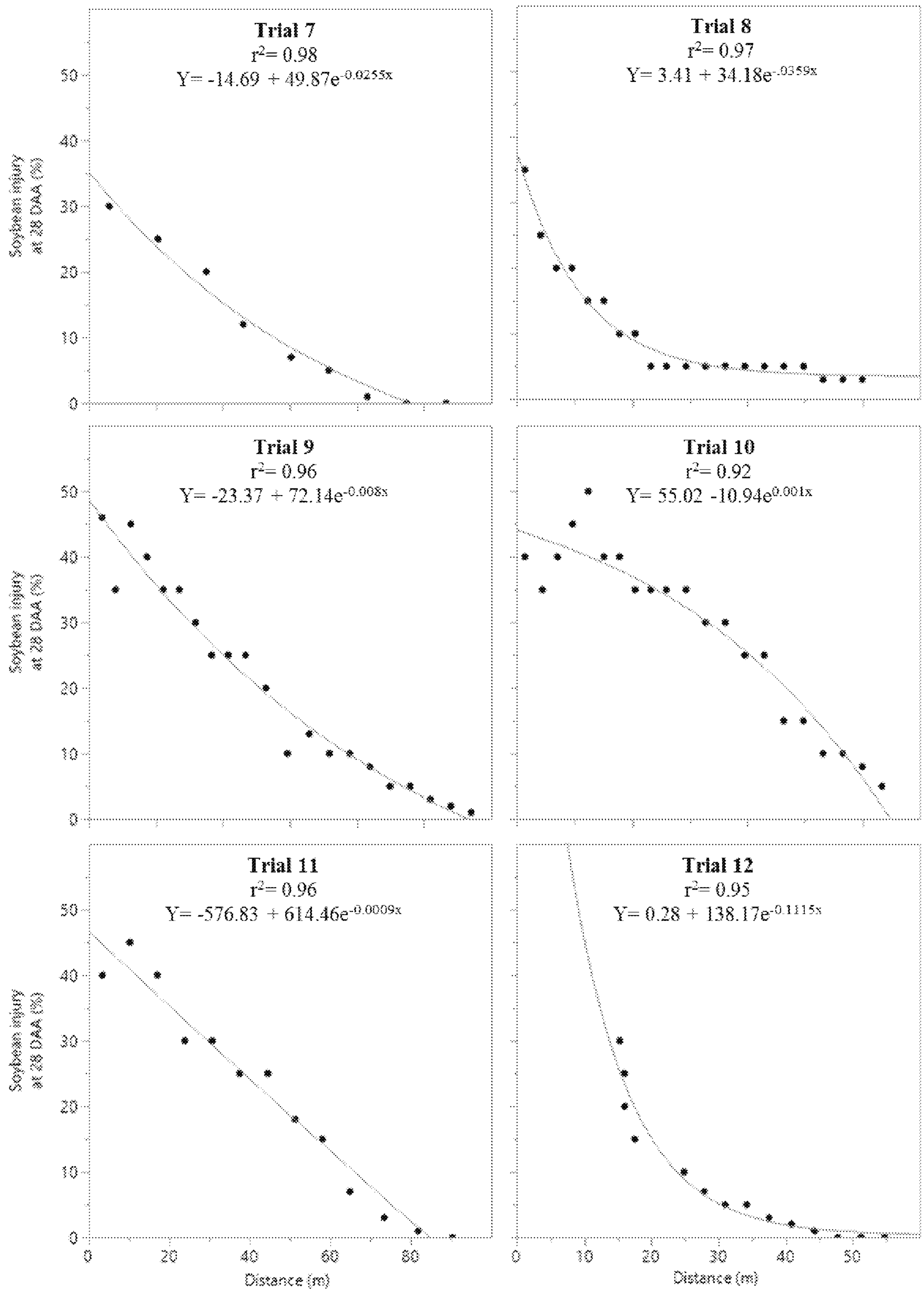


Figure 7. Scatterplot matrix of soybean observations after a diglycolamine dicamba drift event at R6. Measurements at 28 days after application (DAA) were not taken for R6 drift trials due to soybean leaf drop as the crop was approaching maturity. Heights and yield are reported as percentage of the uninjured. Uninjured is referring to the average of three random plots outside of the drift plume that were recorded to have no visual injury at 28 DAA.

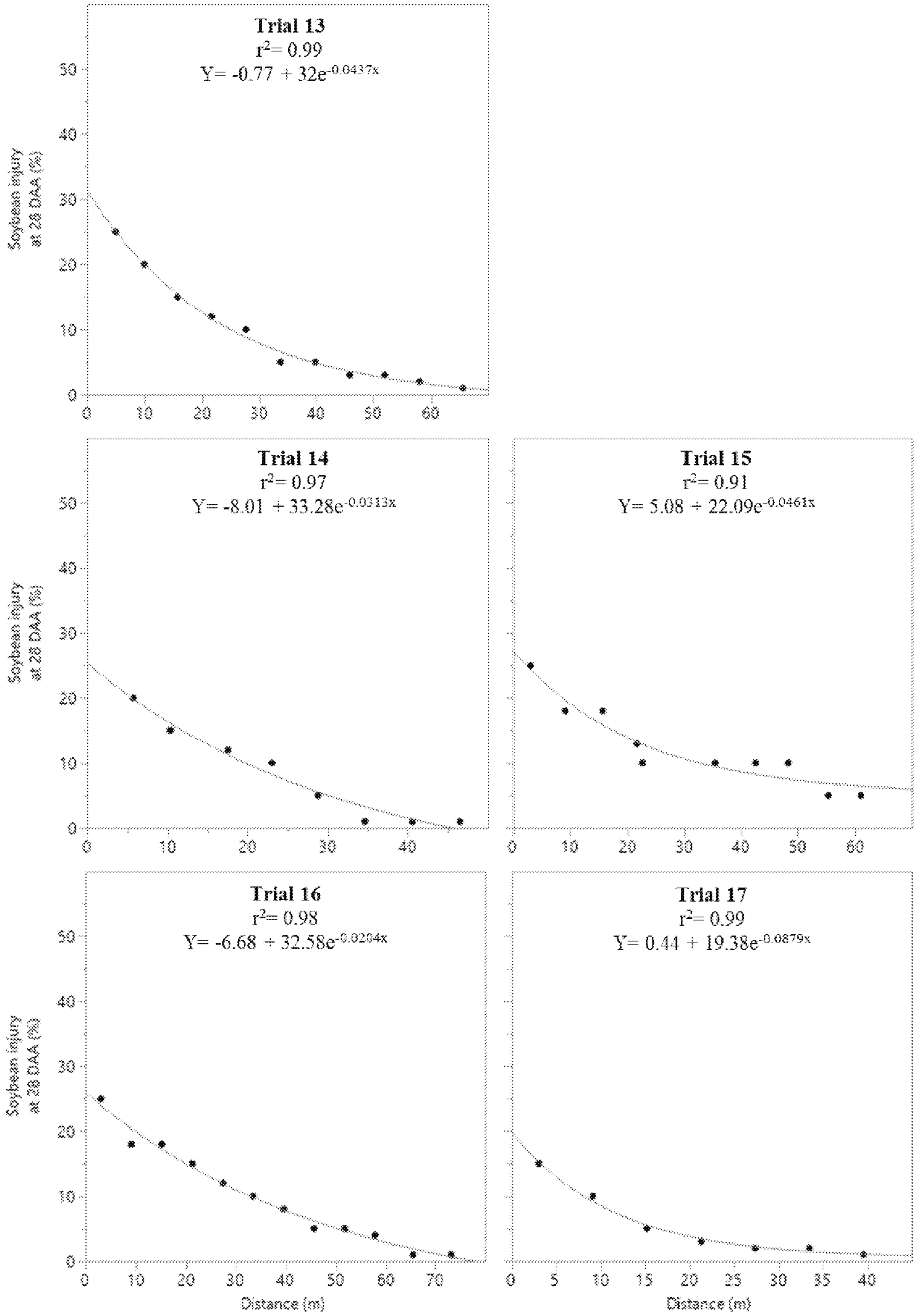
Chapter 1 Appendices



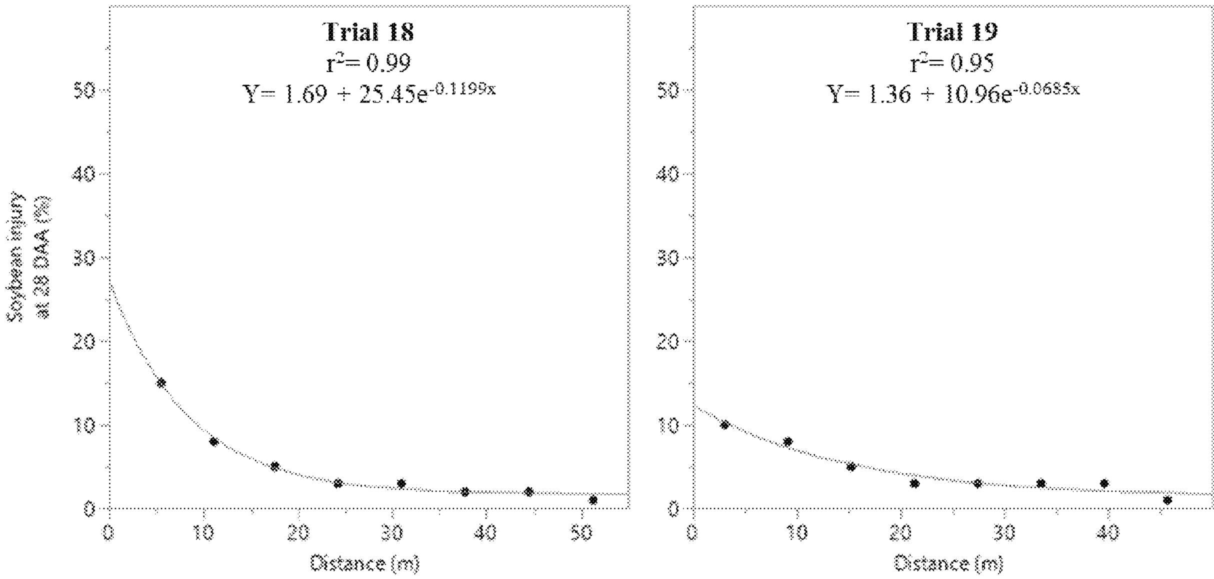
Appendix Figure 1. The relationship between downwind distance and soybean injury at 28 days after application (DAA) for R1 drift events ($\alpha = 0.05$). Soybean injury was rated on a scale from 0 to 100% with 0% being no injury and 100% being plant death.



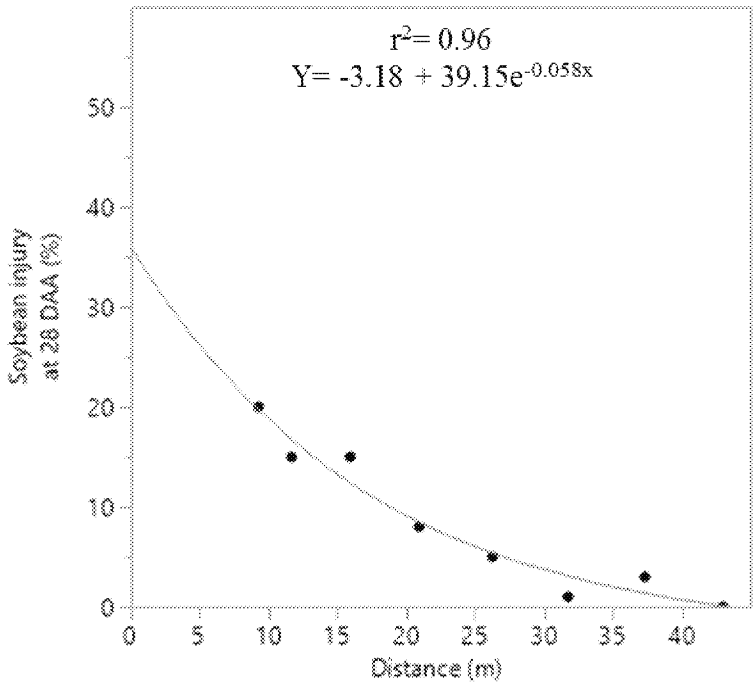
Appendix Figure 2. The relationship between downwind distance and soybean injury at 28 days after application (DAA) for R2 drift events (α= 0.05). Soybean injury was rated on a scale from 0 to 100% with 0% being no injury and 100% being plant death.



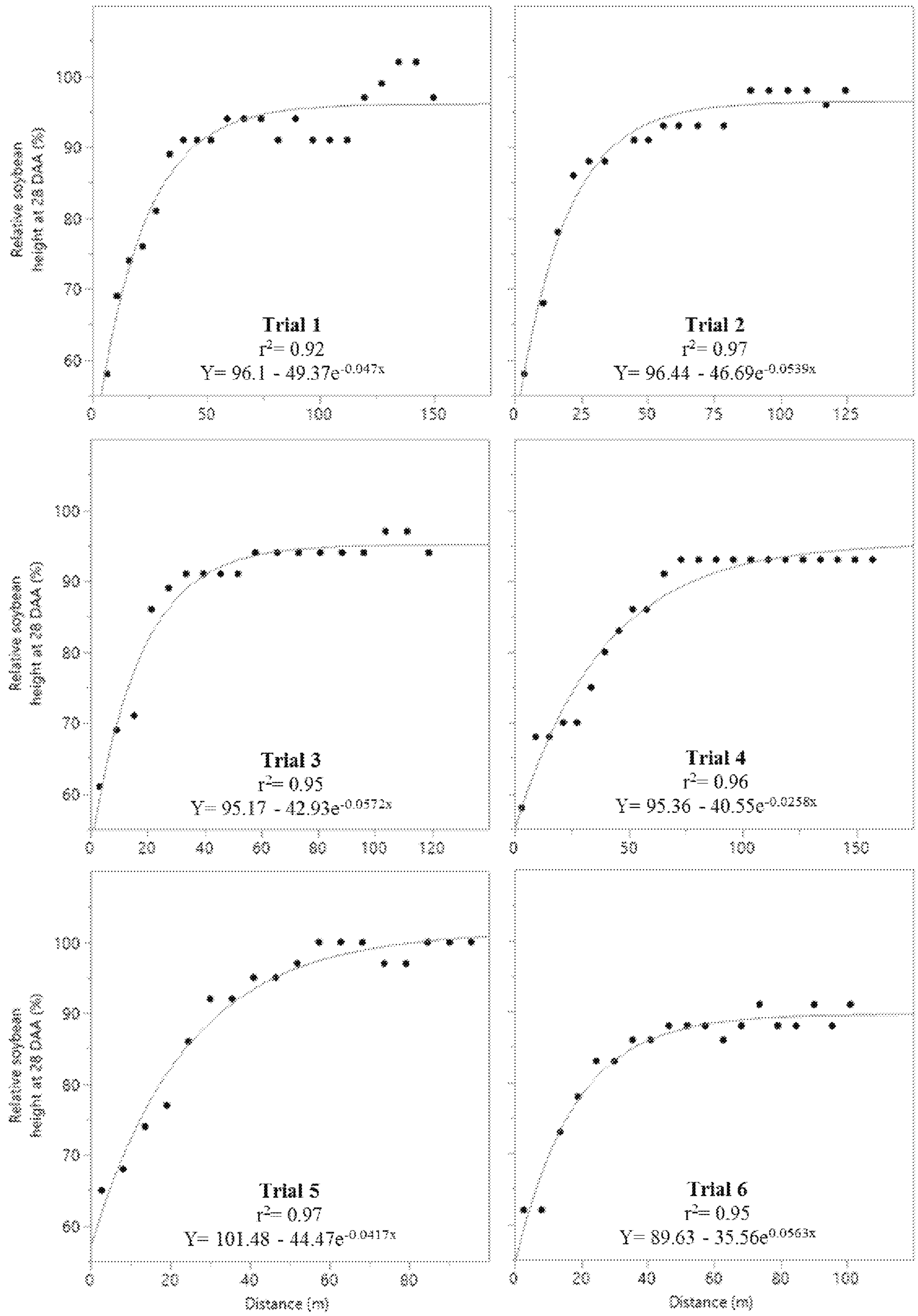
Appendix Figure 3. The relationship between downwind distance and soybean injury at 28 days after application (DAA) for R3 drift events (α= 0.05). Soybean injury was rated on a scale from 0 to 100% with 0% being no injury and 100% being plant death.



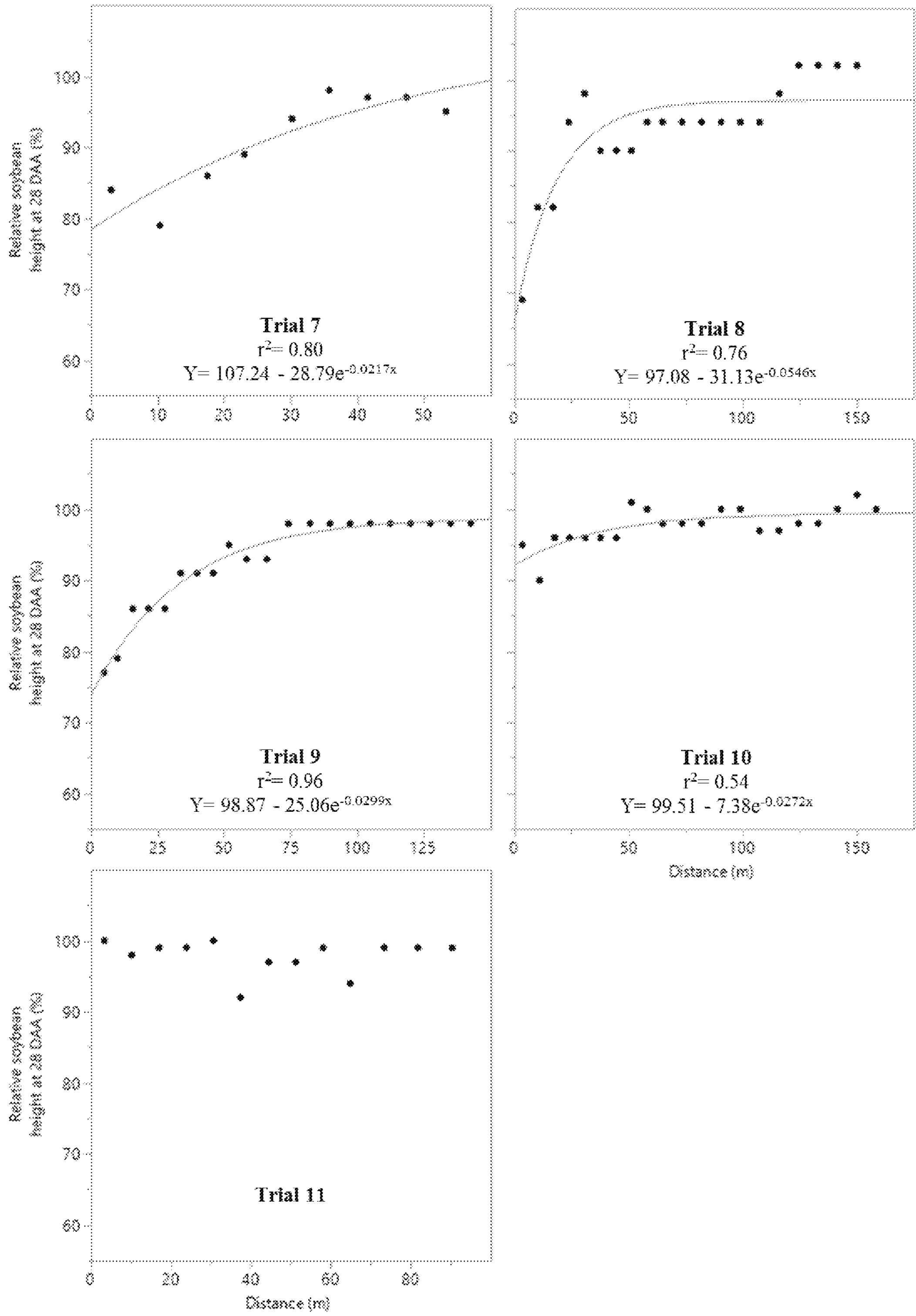
Appendix Figure 4. The relationship between downwind distance and soybean injury at 28 days after application (DAA) for R4 drift events (α= 0.05). Soybean injury was rated on a scale from 0 to 100% with 0% being no injury and 100% being plant death.



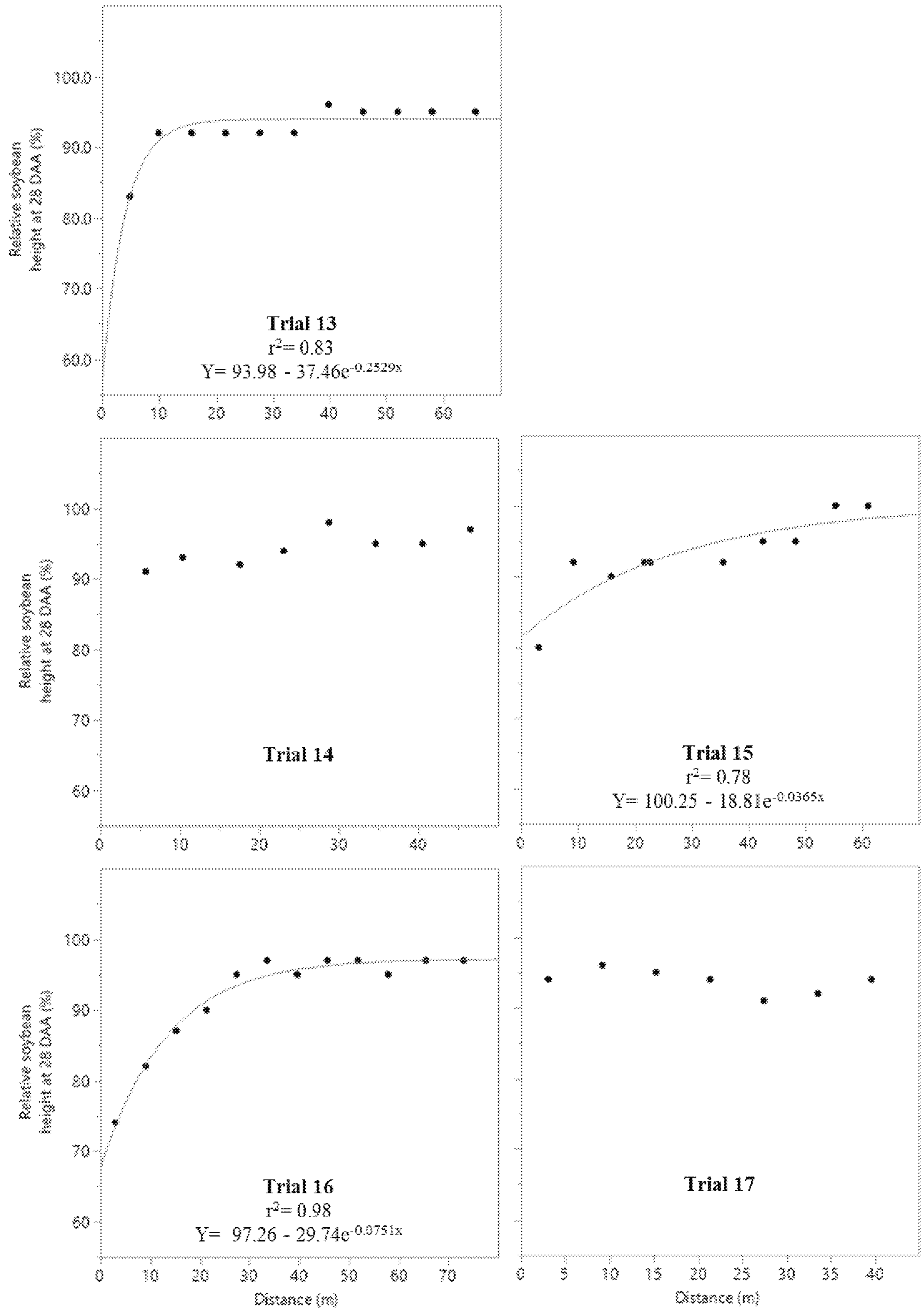
Appendix Figure 5. The relationship between downwind distance and soybean injury for trial 20 (R5) (α= 0.05). Soybean injury was rated on a scale from 0 to 100% with 0% being no injury and 100% being plant death.



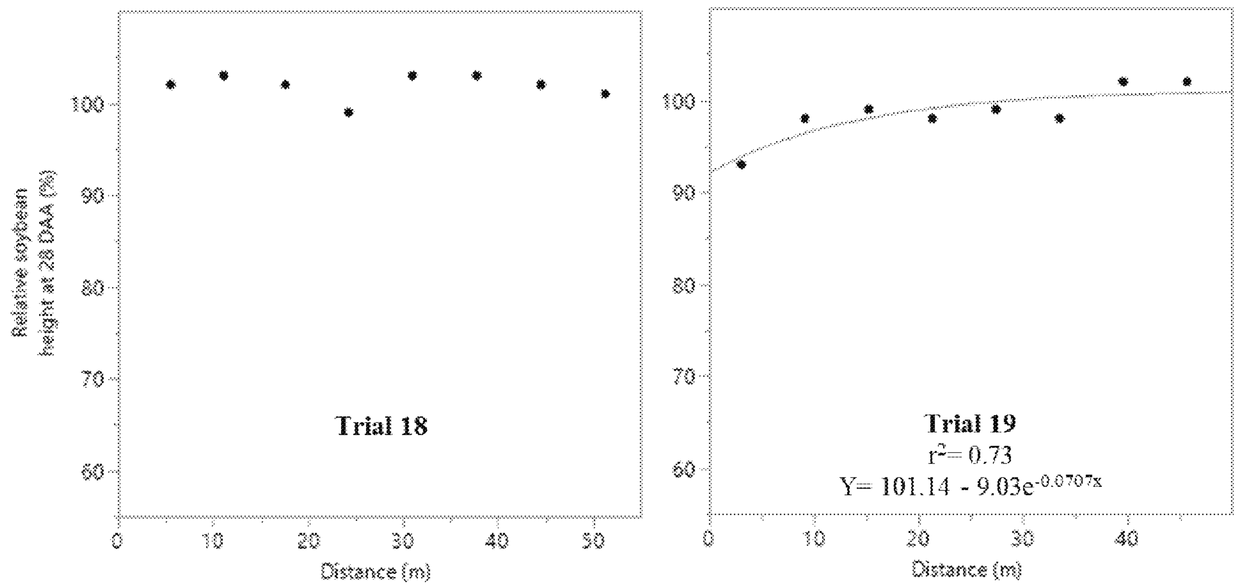
Appendix Figure 6. The relationship between downwind distance and soybean height at 28 days after application (DAA) for R1 drift events (α= 0.05). Soybean height was converted to a percent of the uninjured. The uninjured was the average height at 28 DAA of 3 random plots with no injury at 28 DAA.



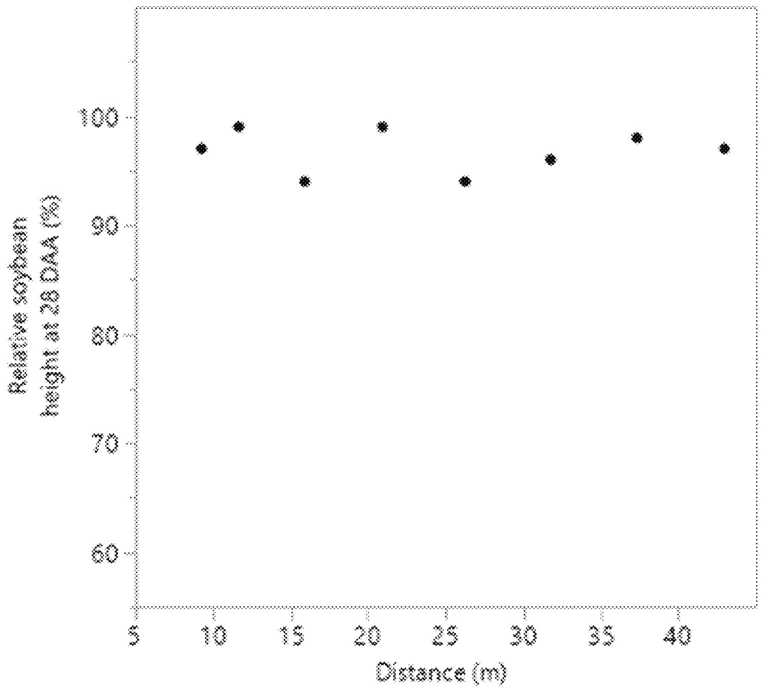
Appendix Figure 7. The relationship between downwind distance and soybean height at 28 days after application (DAA) for R2 drift events (α= 0.05). Soybean height was converted to a percent of the uninjured. The uninjured was the average height at 28 DAA of 3 random plots with no injury at 28 DAA. Trial 11 was not significant. Trial 12 is not shown because height data at 28 DAA was not taken.



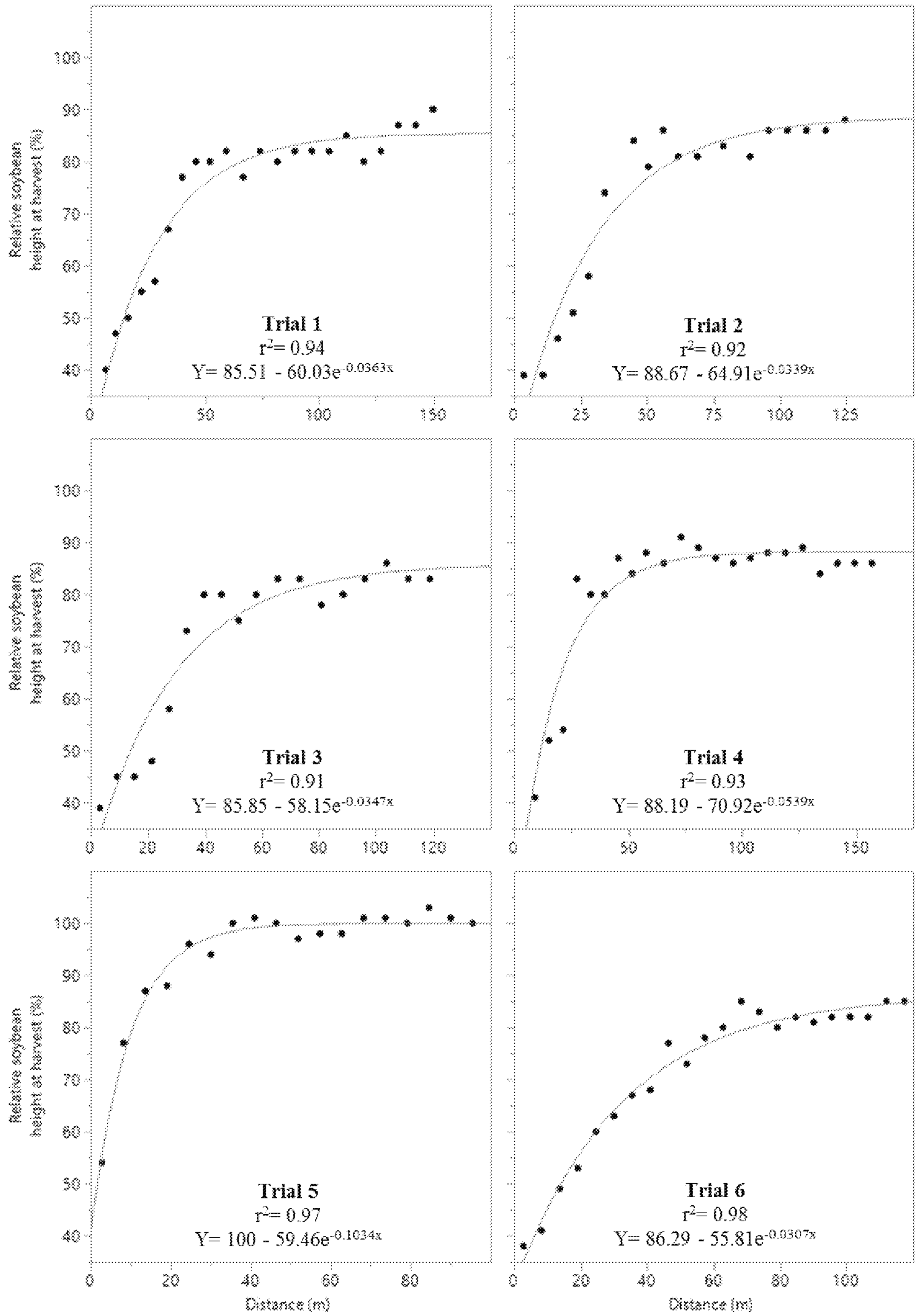
Appendix Figure 8. The relationship between downwind distance and soybean height at 28 days after application (DAA) for R3 drift events (α= 0.05). Soybean height was converted to a percent of the uninjured. The uninjured was the average height at 28 DAA of 3 random plots with no injury at 28 DAA. Trials 14 and 17 were not significant.



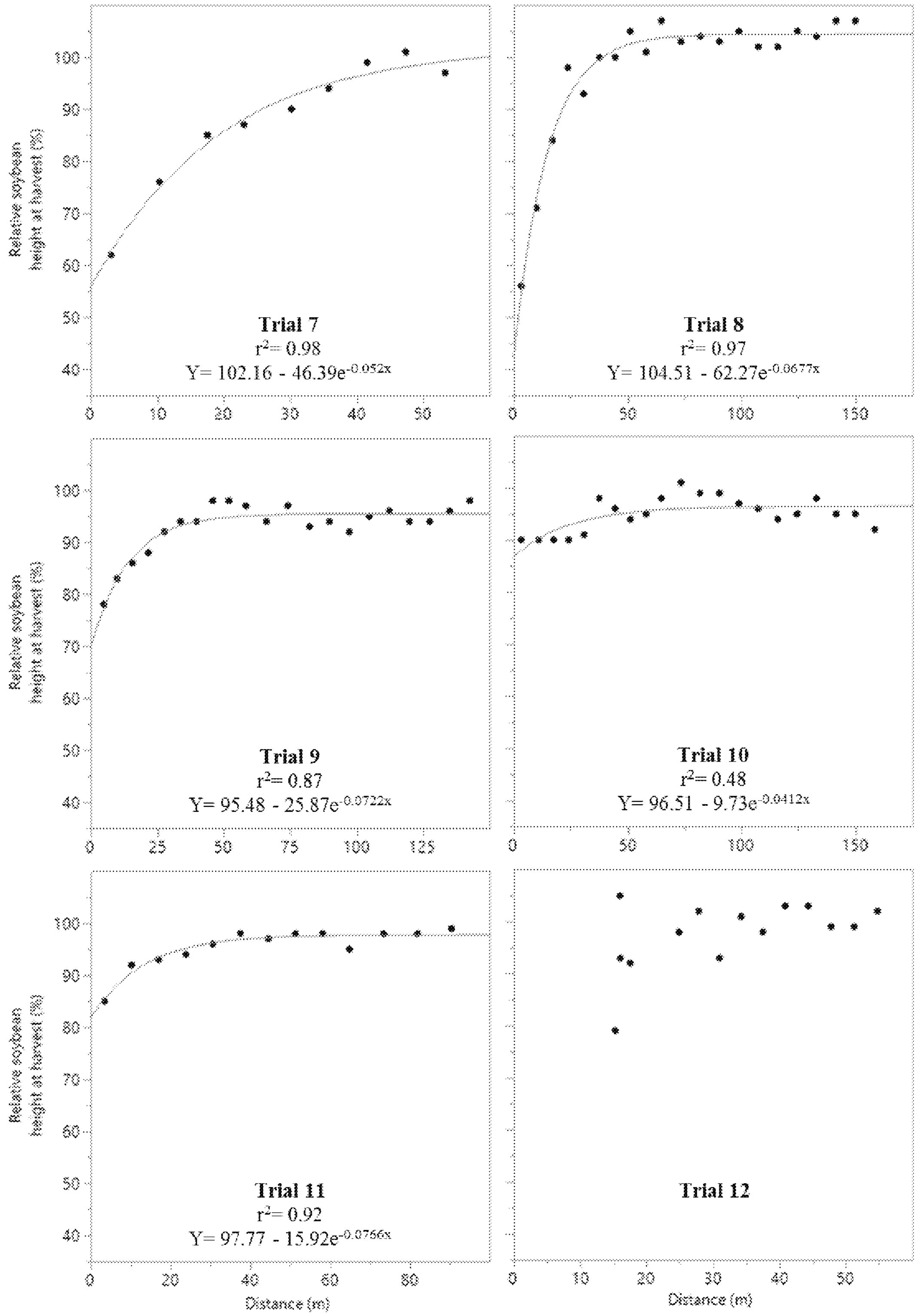
Appendix Figure 9. The relationship between downwind distance and soybean height at 28 days after application (DAA) for R4 drift events (α= 0.05). Soybean height was converted to a percent of the uninjured. The uninjured was the average height at 28 DAA of 3 random plots with no injury at 28 DAA. Trial 18 was not significant.



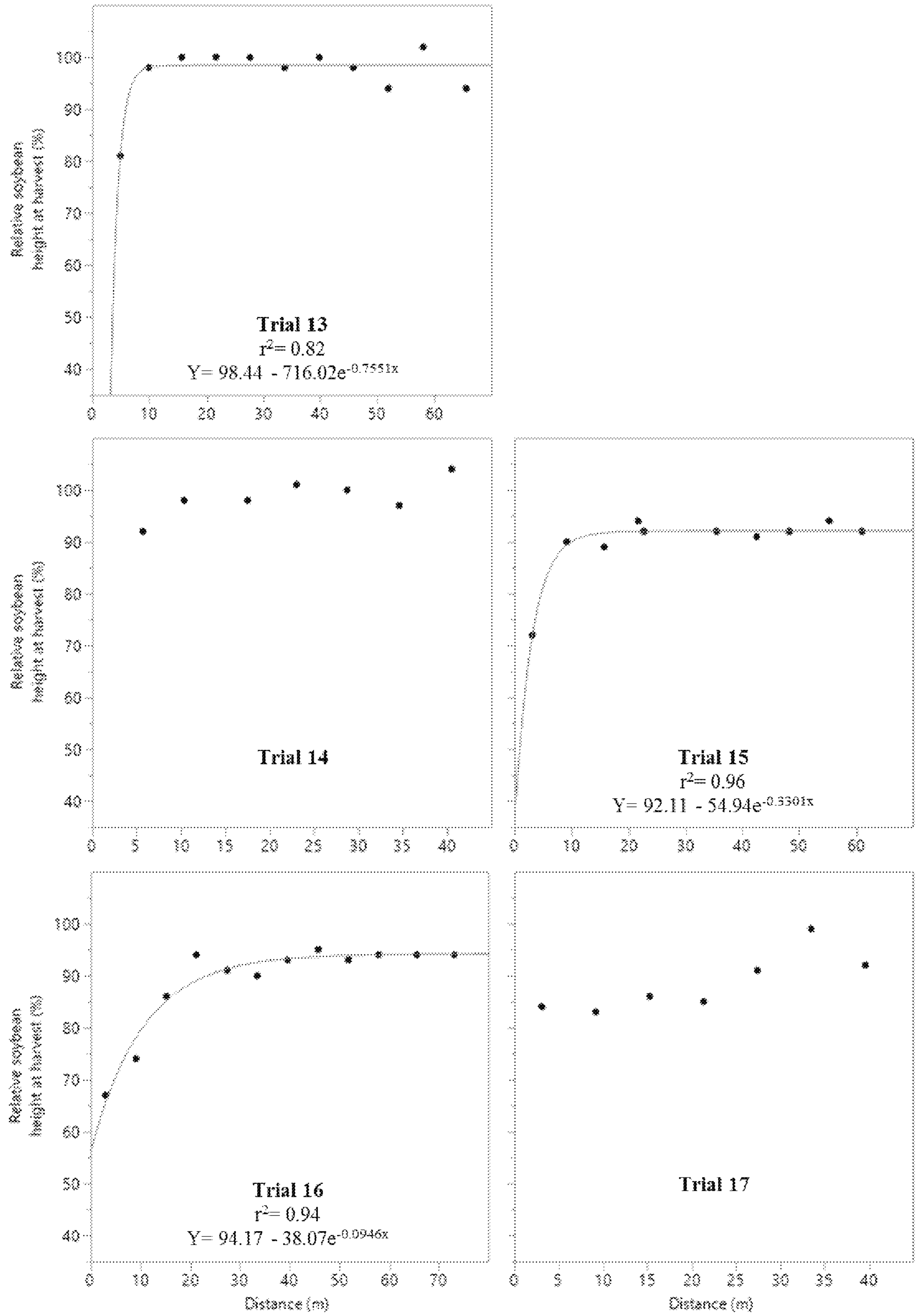
Appendix Figure 10. The relationship between downwind distance and height at 28 days after application (DAA) for trial 20 (R5) (α= 0.05). Soybean height was converted to a percent of the uninjured. The uninjured was the average height at 28 DAA of 3 random plots with no injury at 28 DAA. Trial 20 was not significant.



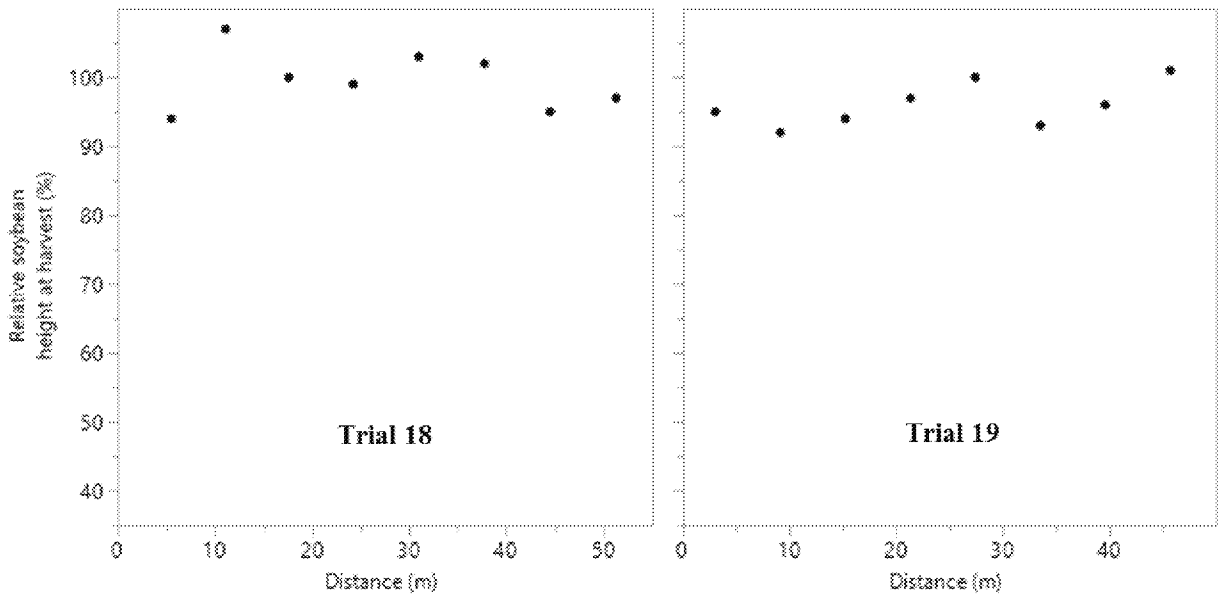
Appendix Figure 11. The relationship between downwind distance and soybean height at maturity for R1 drift events (α= 0.05). Soybean height was converted to a percent of the uninjured. The uninjured was the average height at maturity of 3 random plots with no injury at 28 DAA.



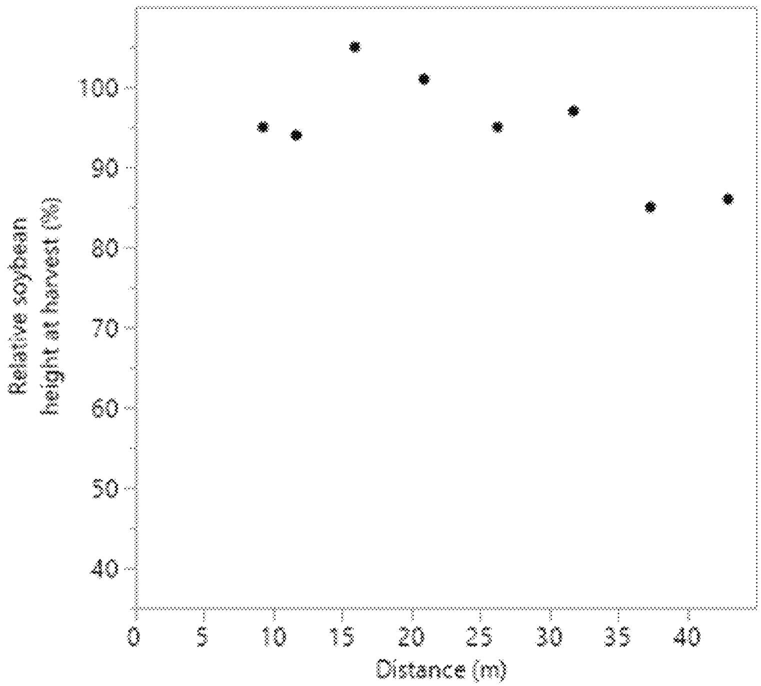
Appendix Figure 12. The relationship between downwind distance and soybean height at maturity for R2 drift events (α= 0.05). Soybean height was converted to a percent of the uninjured. The uninjured was the average height at maturity of 3 random plots with no injury at 28 DAA. Trial 12 was not significant.



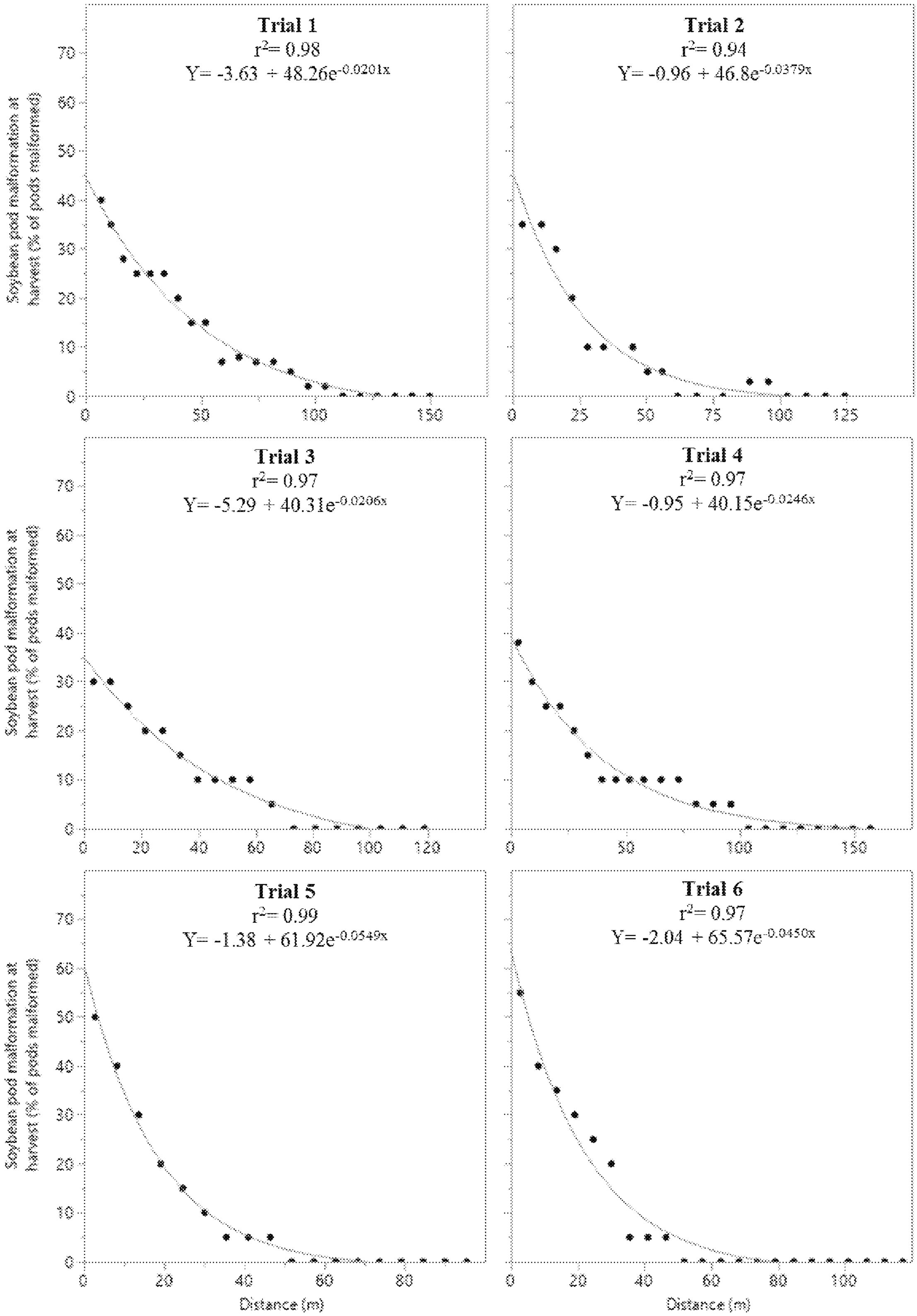
Appendix Figure 13. The relationship between downwind distance and soybean height at maturity for R3 drift events (α= 0.05). Soybean height was converted to a percent of the uninjured. The uninjured was the average height at maturity of 3 random plots with no injury at 28 DAA. Trials 14 and 17 were not significant.



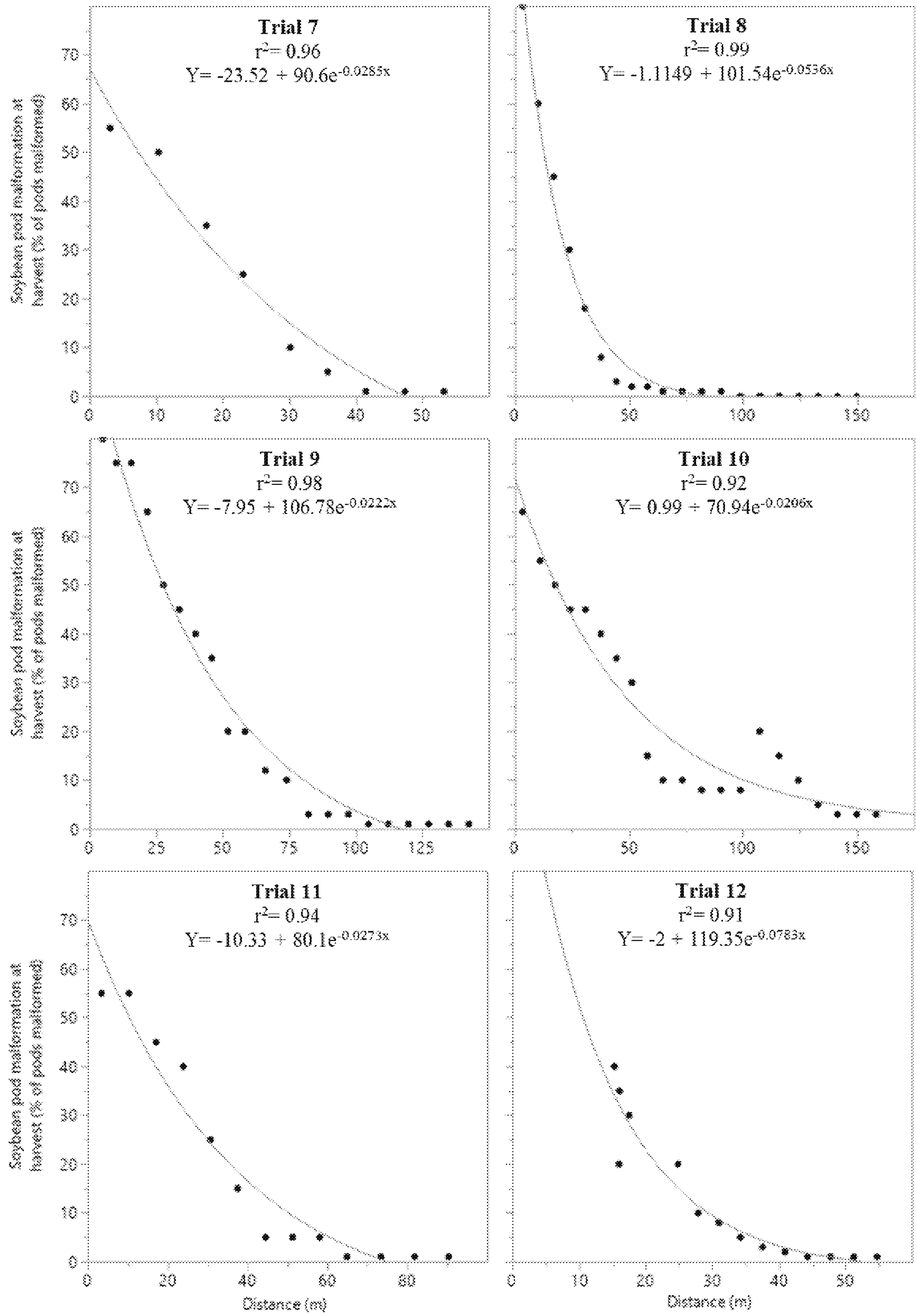
Appendix Figure 14. The relationship between downwind distance and soybean height at maturity for R4 drift events (α= 0.05). Soybean height was converted to a percent of the uninjured. The uninjured was the average height at maturity of 3 random plots with no injury at 28 DAA. Neither trial 18 or 19 were significant.



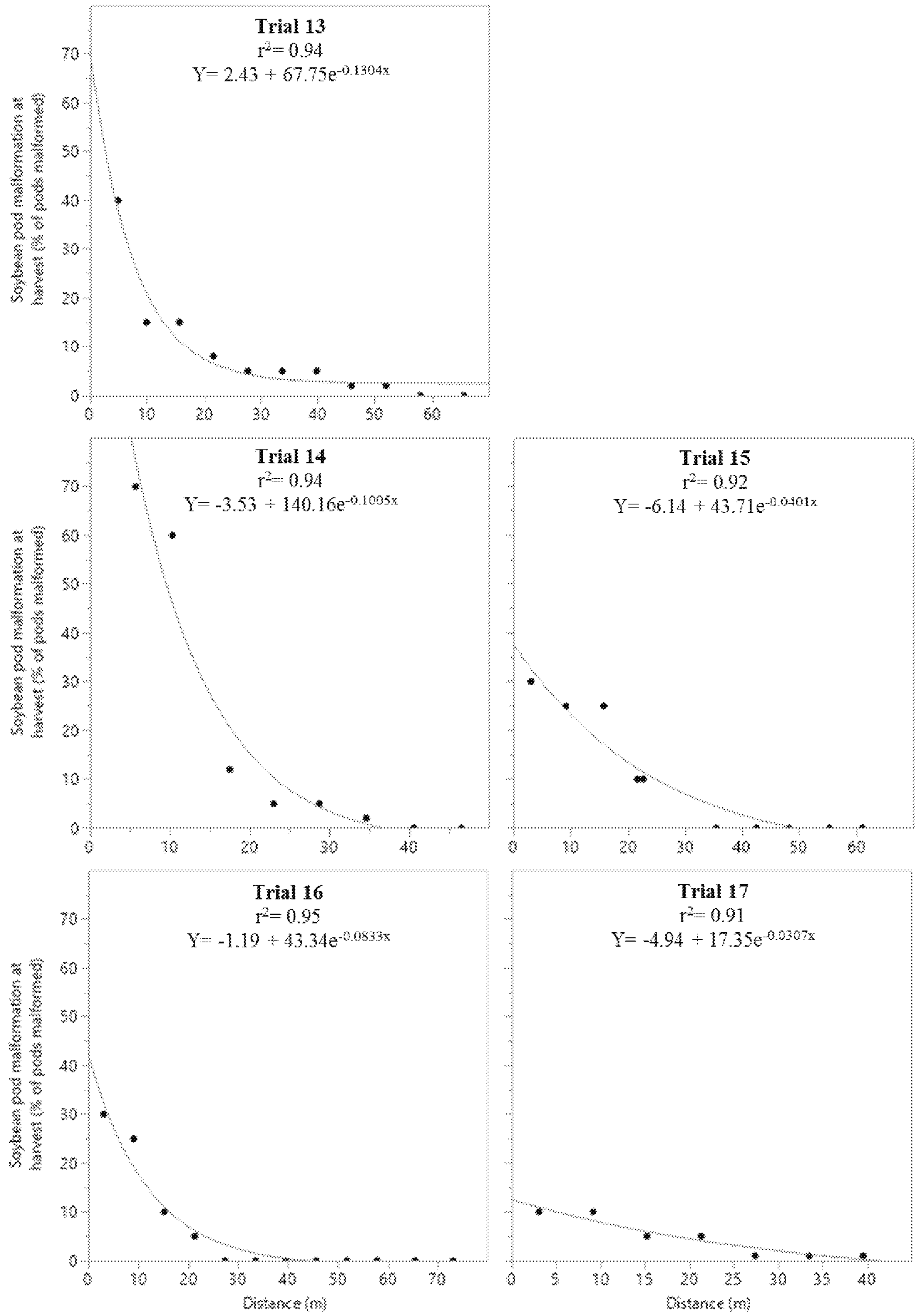
Appendix Figure 15. The relationship between downwind distance and soybean height at harvest for trial 20 (R5) (α= 0.05). Soybean height was converted to a percent of the uninjured. The uninjured was the average harvest height of 3 random plots with no injury at 28 DAA. Trial 20 was not significant.



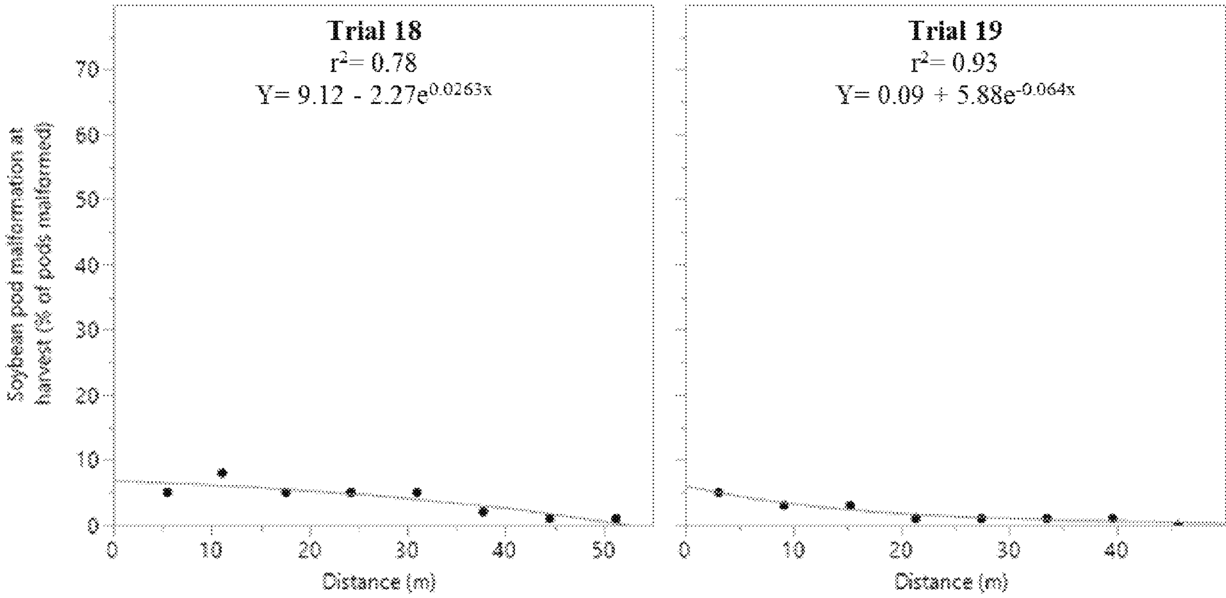
Appendix Figure 16. The relationship between downwind distance and soybean pod malformation at maturity for R1 drift events (α= 0.05). Soybean pod malformation was rated as a percent of the total pods malformed.



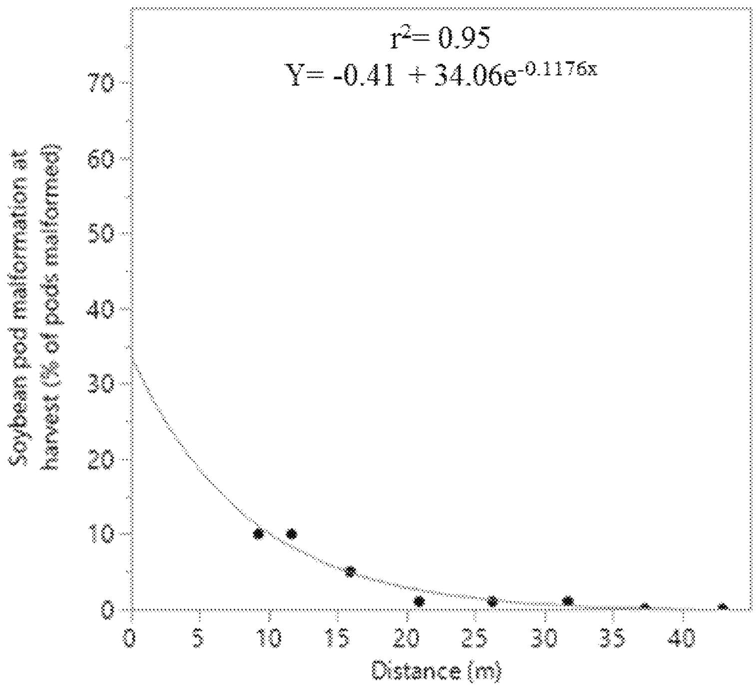
Appendix Figure 17. The relationship between downwind distance and soybean pod malformation at maturity for R2 drift events (α= 0.05). Soybean pod malformation was rated as a percent of the total pods malformed.



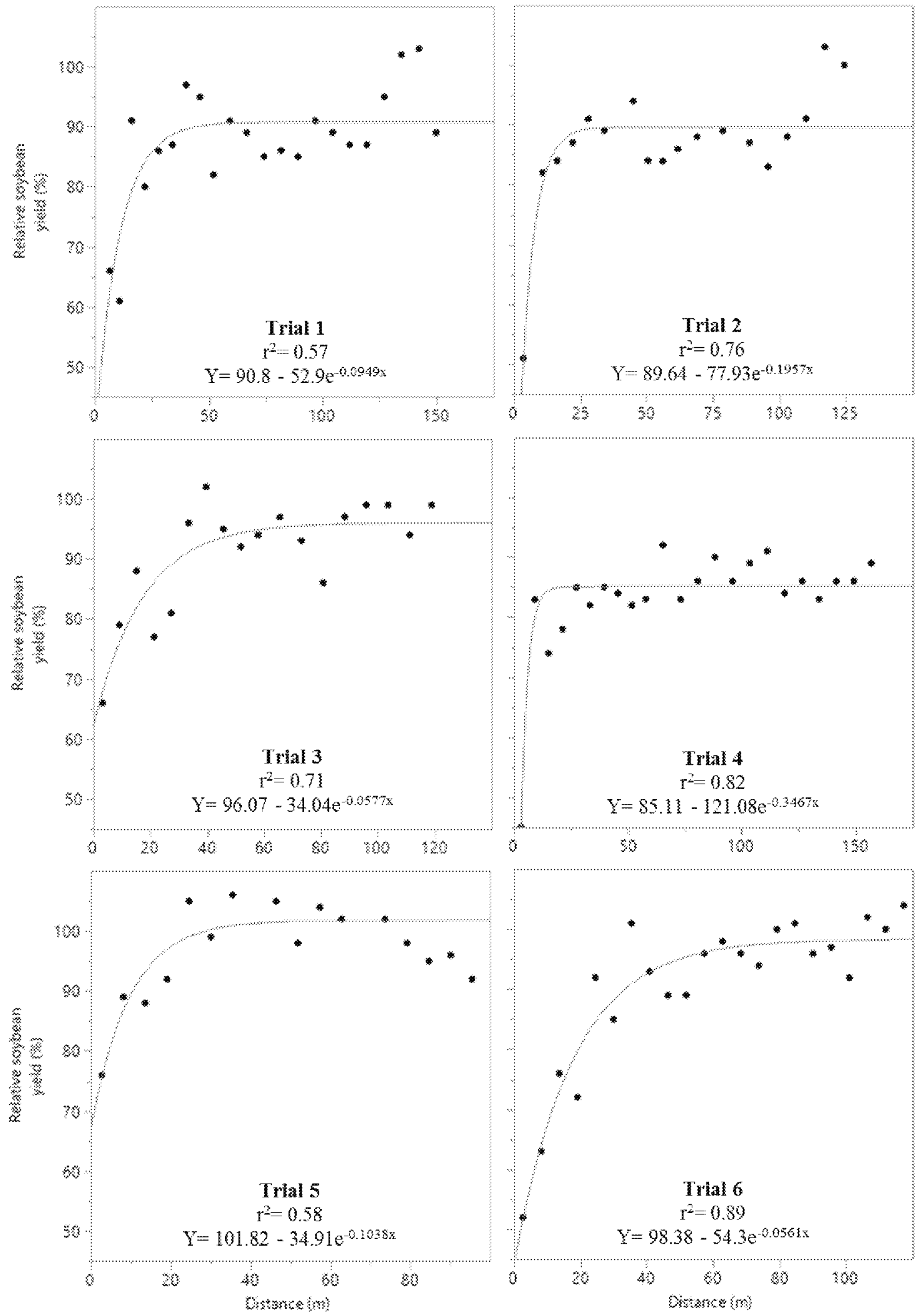
Appendix Figure 18. The relationship between downwind distance and soybean pod malformation at maturity for R3 drift events ($\alpha = 0.05$). Soybean pod malformation was rated as a percent of the total pods malformed.



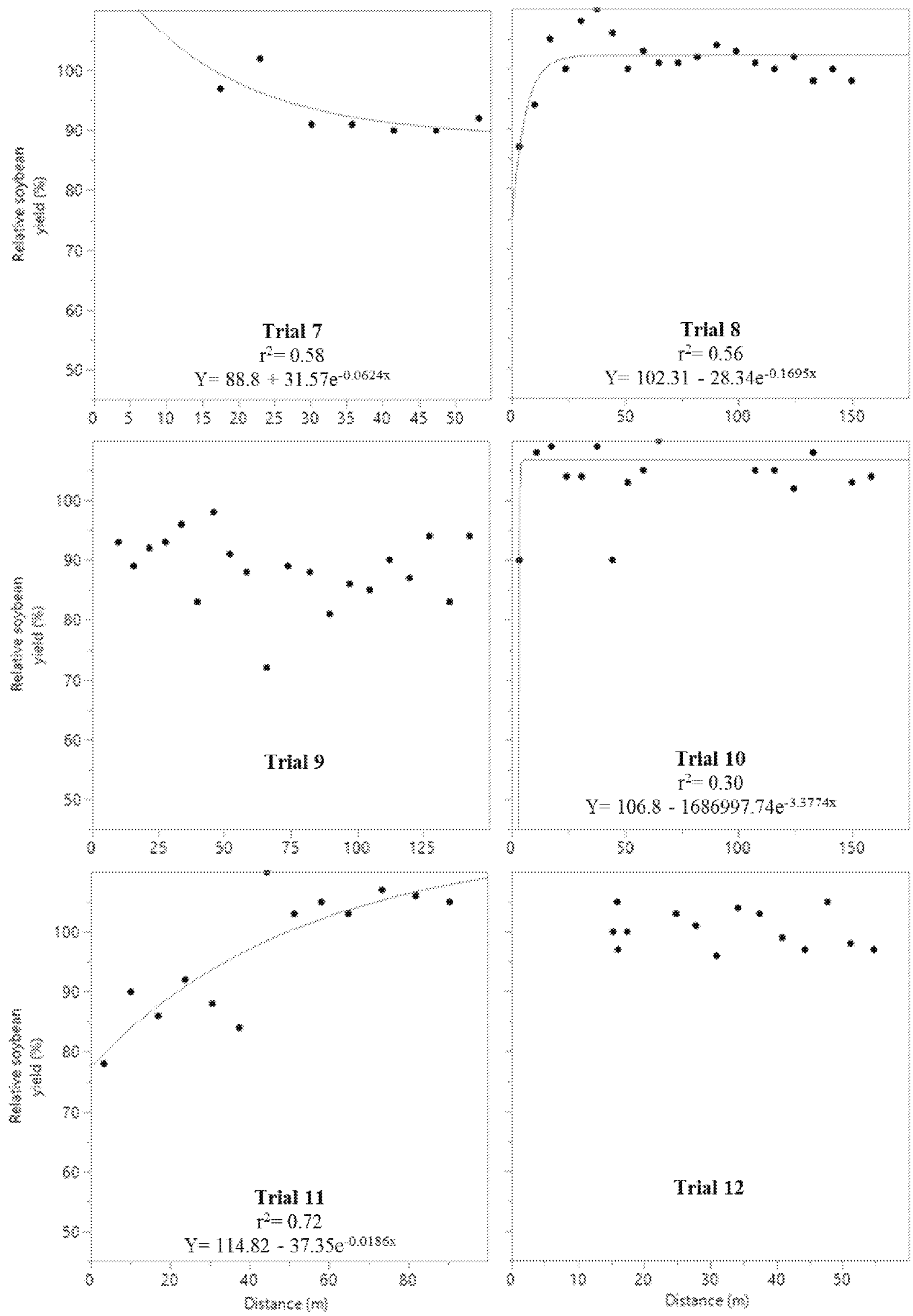
Appendix Figure 19. The relationship between downwind distance and soybean pod malformation at maturity for R4 drift events (α= 0.05). Soybean pod malformation was rated as a percent of the total pods malformed.



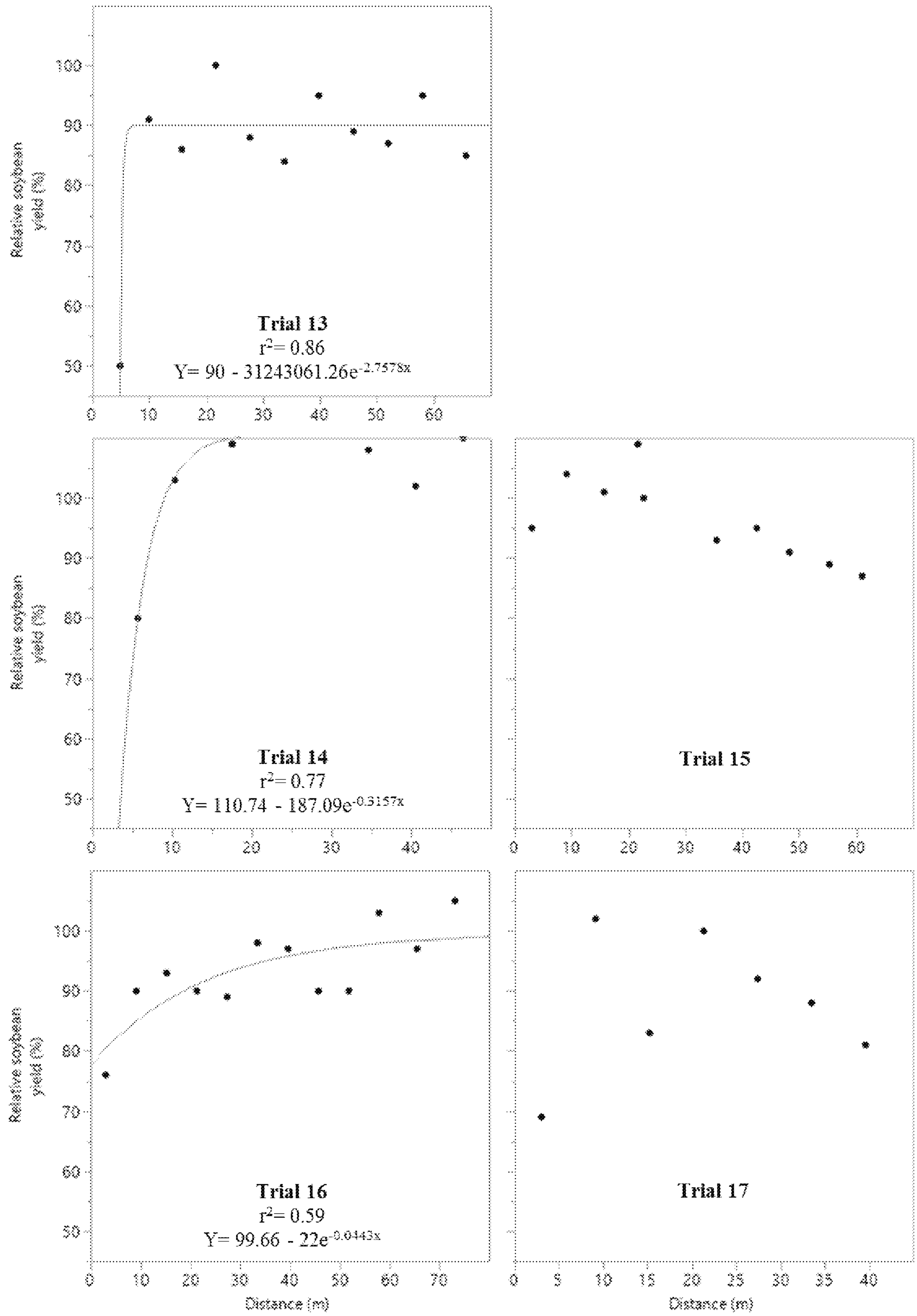
Appendix Figure 20. The relationship between downwind distance and soybean pod malformation for trial 20 (R5) ($\alpha = 0.05$). Soybean pod malformation was rated as a percent of the total pods malformed.



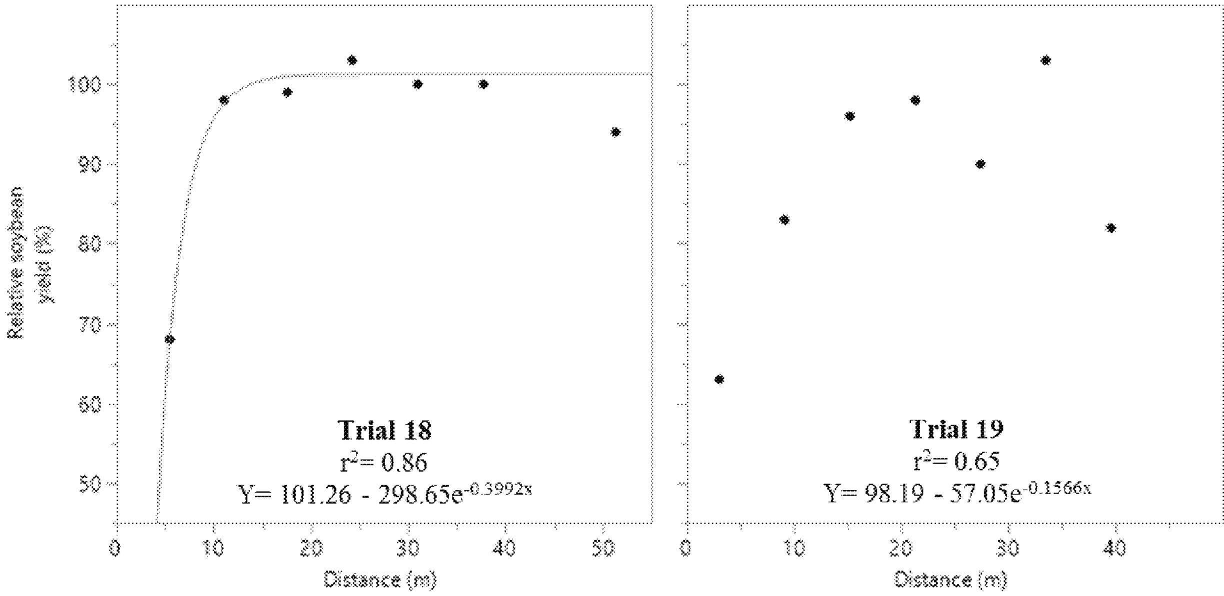
Appendix Figure 21. The relationship between downwind distance and soybean yield for R1 drift events (α= 0.05). Soybean yield was converted to a percent of the uninjured. The uninjured was the average yield of 3 random plots within each trial with no injury at 28 DAA.



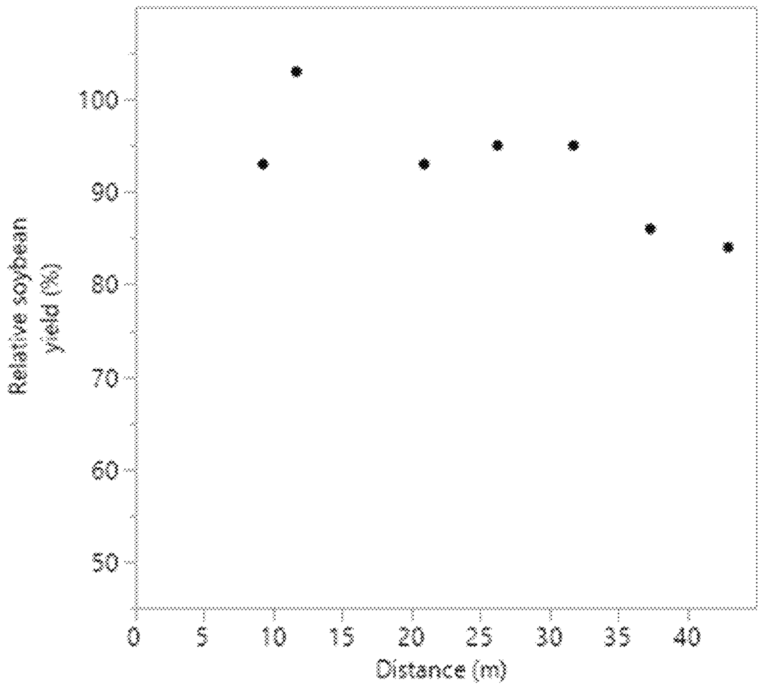
Appendix Figure 22. The relationship between downwind distance and soybean yield for R2 drift events (α= 0.05). Soybean yield was converted to a percent of the uninjured. The uninjured was the average yield of 3 random plots within each trial with no injury at 28 DAA. Trials 9 and 12 were not significant.



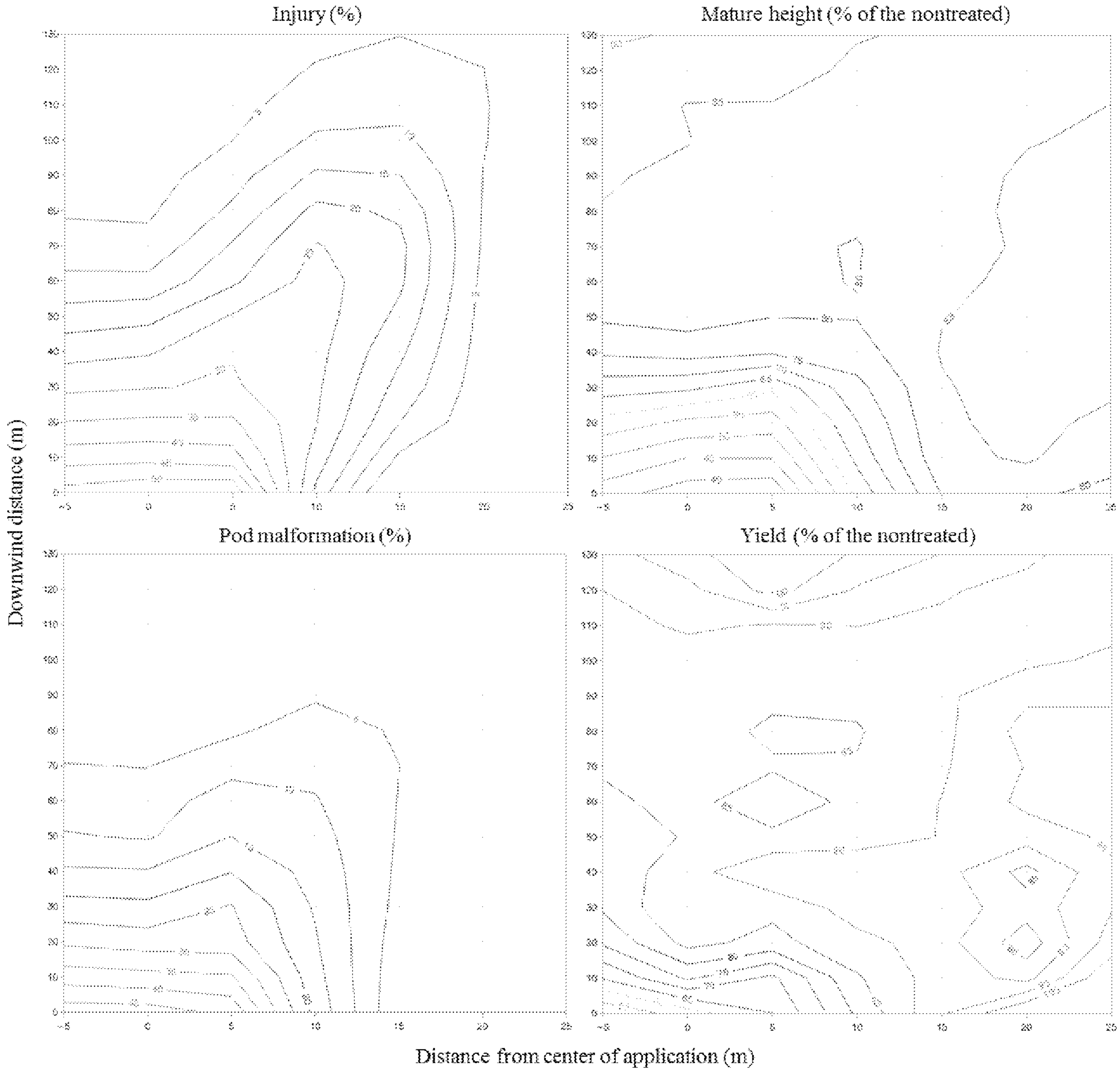
Appendix Figure 23. The relationship between downwind distance and soybean yield for R3 drift events (α= 0.05). Soybean yield was converted to a percent of the uninjured. The uninjured was the average yield of 3 random plots within each trial with no injury at 28 DAA. Trials 15 and 17 were not significant.



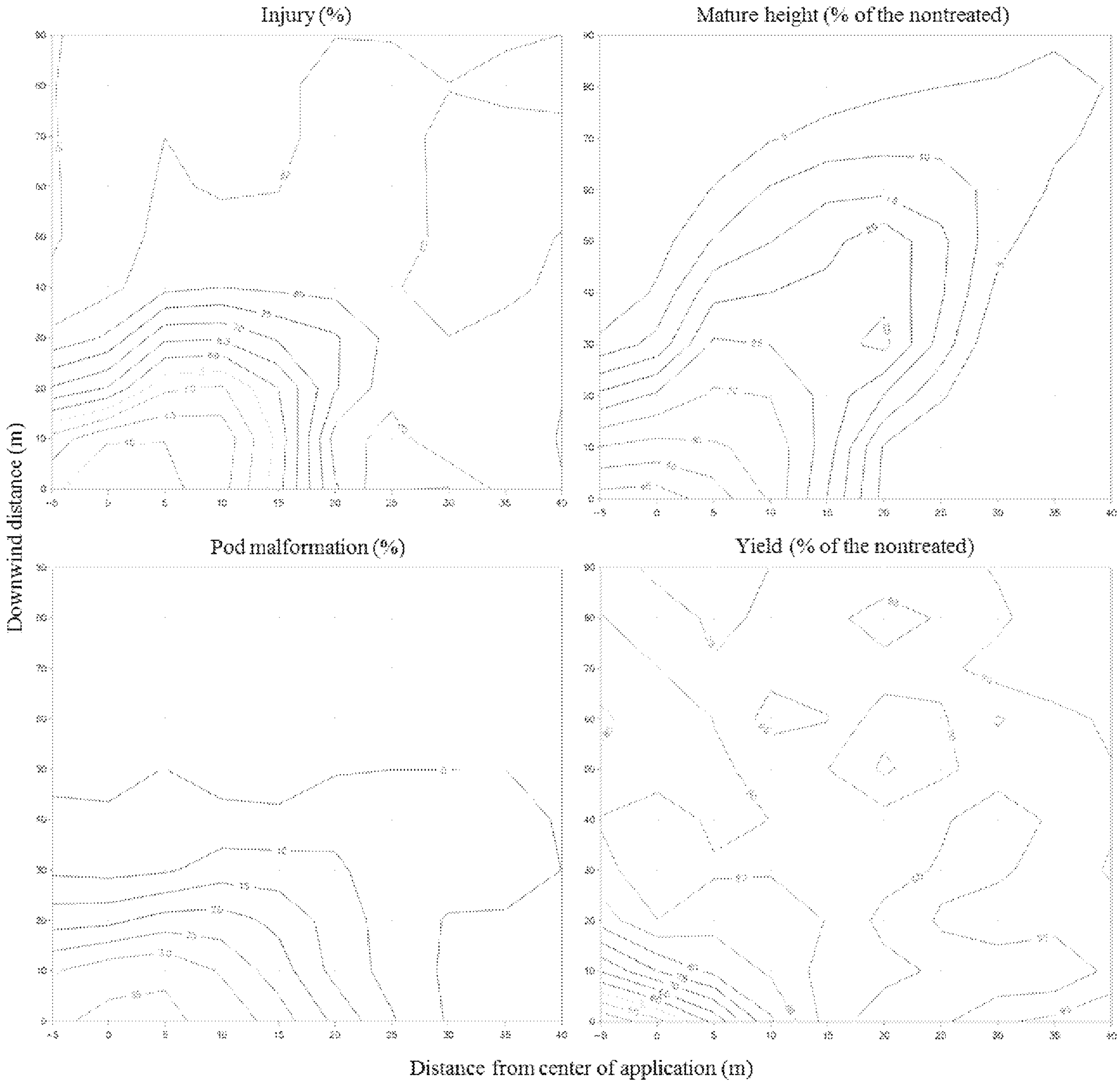
Appendix Figure 24. The relationship between downwind distance and soybean yield for R4 drift events ($\alpha = 0.05$). Soybean yield was converted to a percent of the uninjured. The uninjured was the average yield of 3 random plots within each trial with no injury at 28 DAA. Trial 19 was not significant.



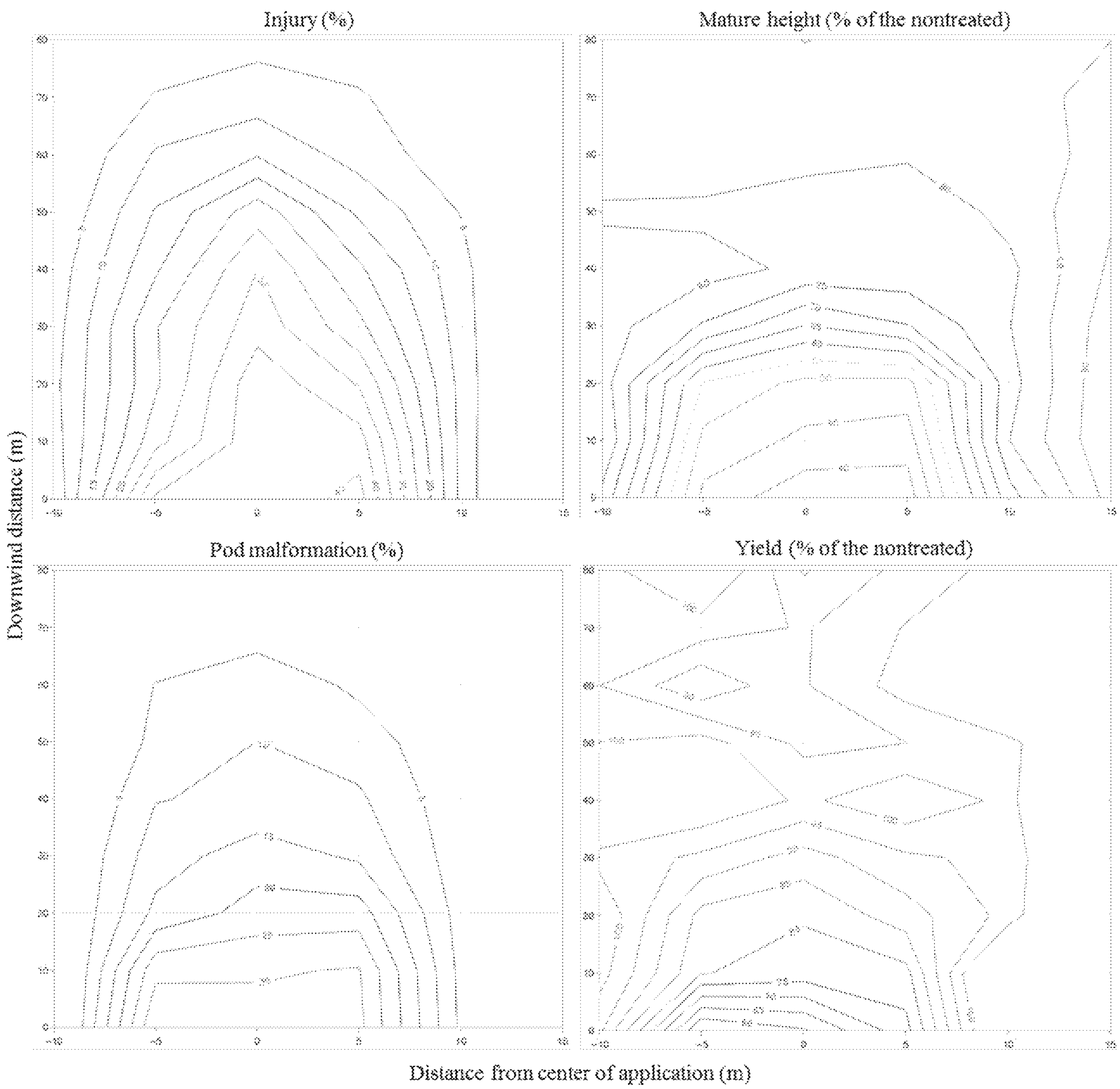
Appendix Figure 25. The relationship between downwind distance and soybean yield for trial 20 (R5) (α= 0.05). Soybean yield was converted to a percent of the uninjured. The uninjured was the average yield of 3 random plots within each trial with no injury at 28 DAA. Trial 20 was not significant.



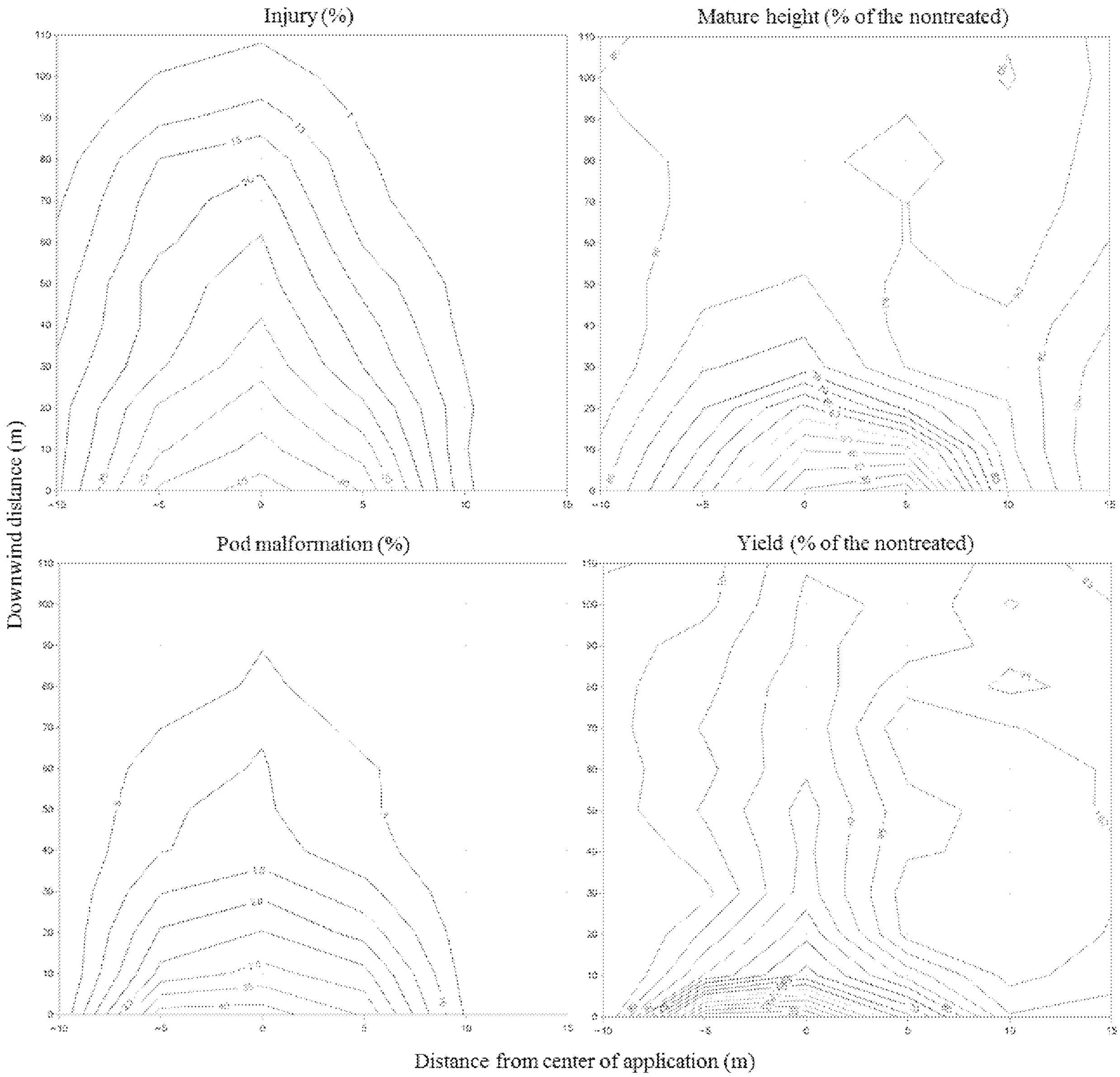
Appendix figure 26. Contour maps illustrating soybean injury, mature height, pod malformation, and yield for trial 1. Soybean injury was rated on a scale from 0 to 100% with 0% being no injury and 100% being plant death. Pod malformation is presented as a percent of total pods malformed. The untreated is the average mature height or yield of 3 random plots within the trial (but outside the drift plume) observed to have no injury at 28 days after application (DAA).



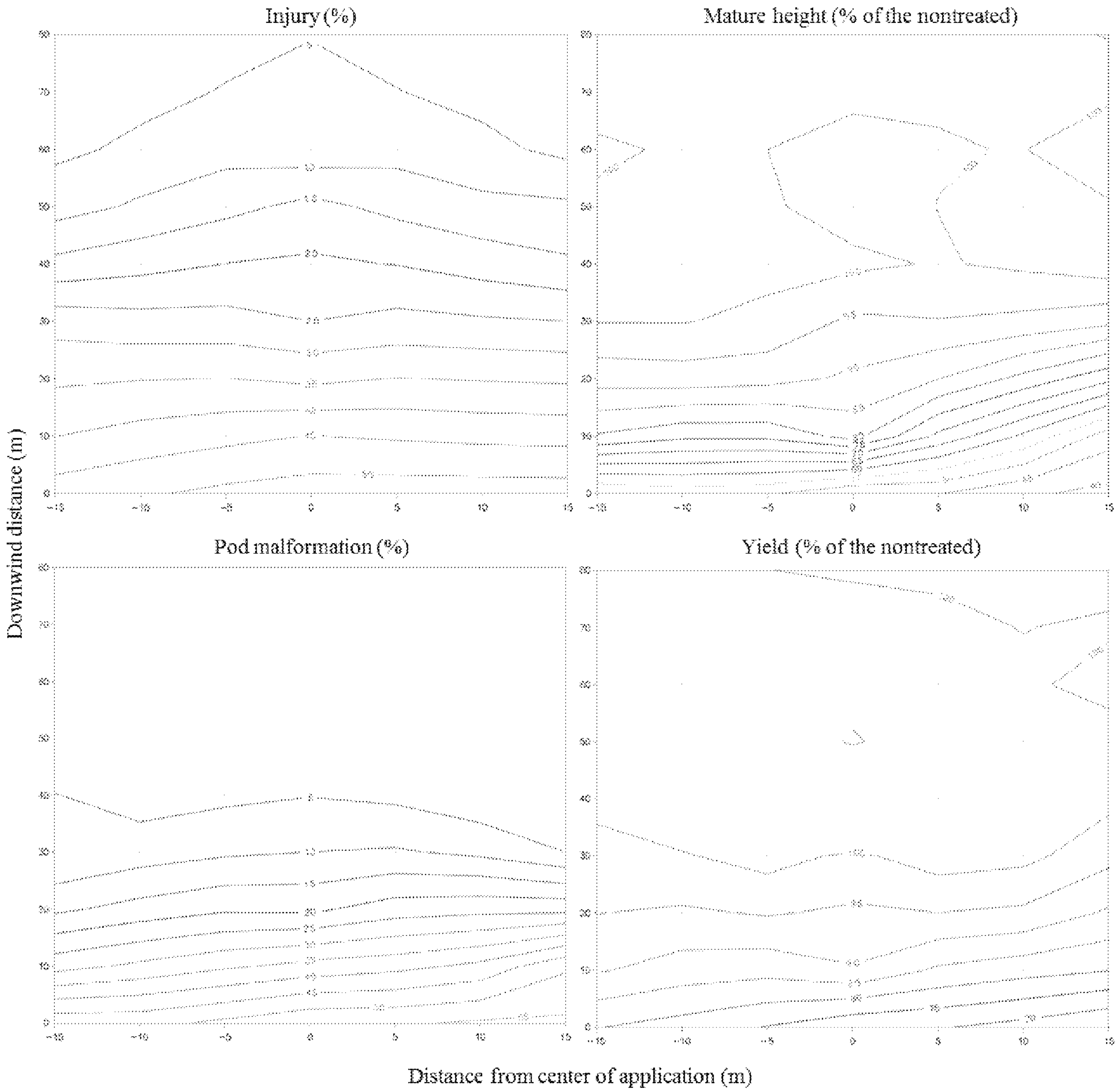
Appendix figure 27. Contour maps illustrating soybean injury, mature height, pod malformation, and yield for trial 2. Soybean injury was rated on a scale from 0 to 100% with 0% being no injury and 100% being plant death. Pod malformation is presented as a percent of total pods malformed. The untreated is the average mature height or yield of 3 random plots within the trial (but outside the drift plume) observed to have no injury at 28 days after application (DAA).



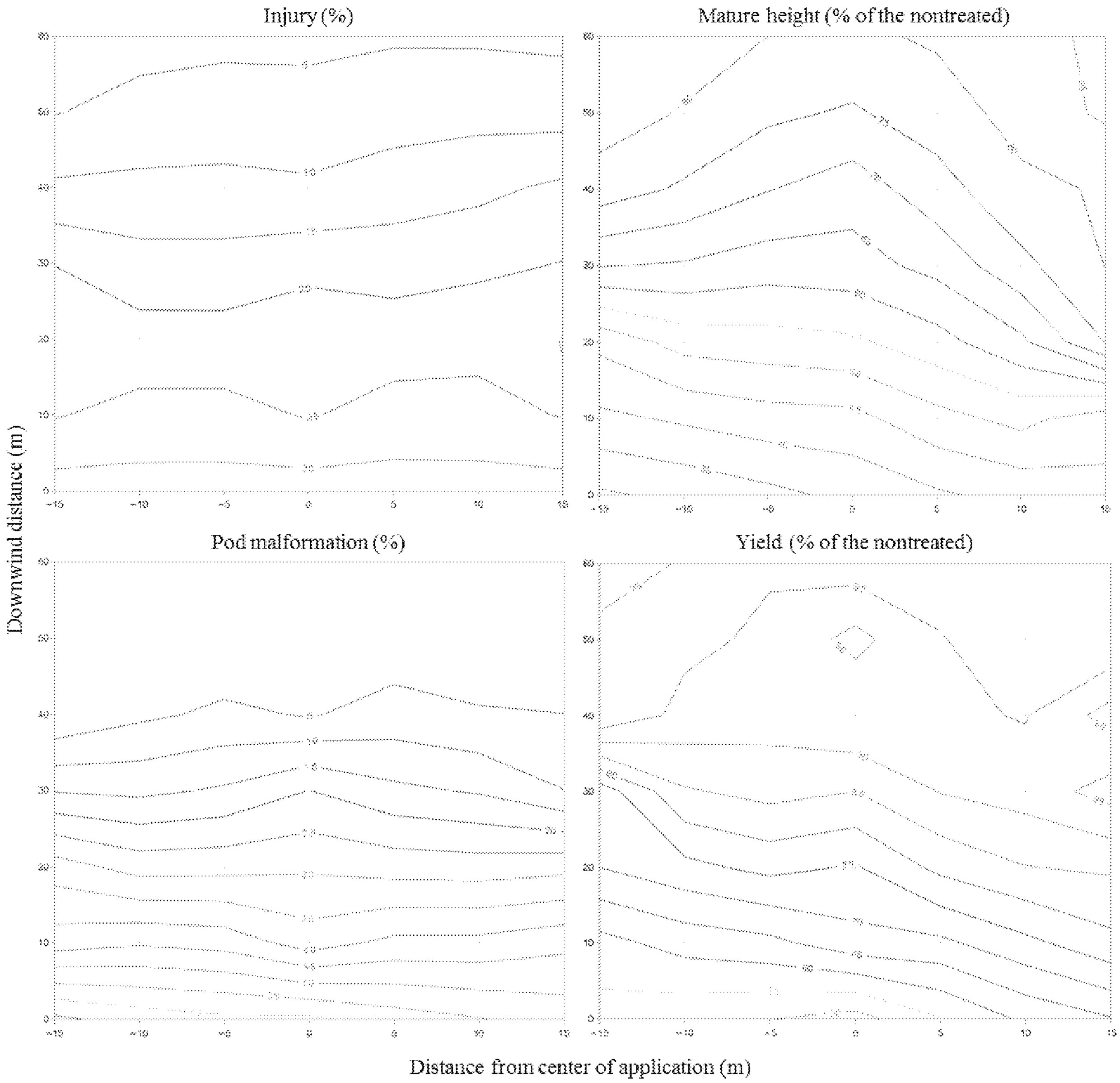
Appendix figure 28. Contour maps illustrating soybean injury, mature height, pod malformation, and yield for trial 3. Soybean injury was rated on a scale from 0 to 100% with 0% being no injury and 100% being plant death. Pod malformation is presented as a percent of total pods malformed. The untreated is the average mature height or yield of 3 random plots within the trial (but outside the drift plume) observed to have no injury at 28 days after application (DAA).



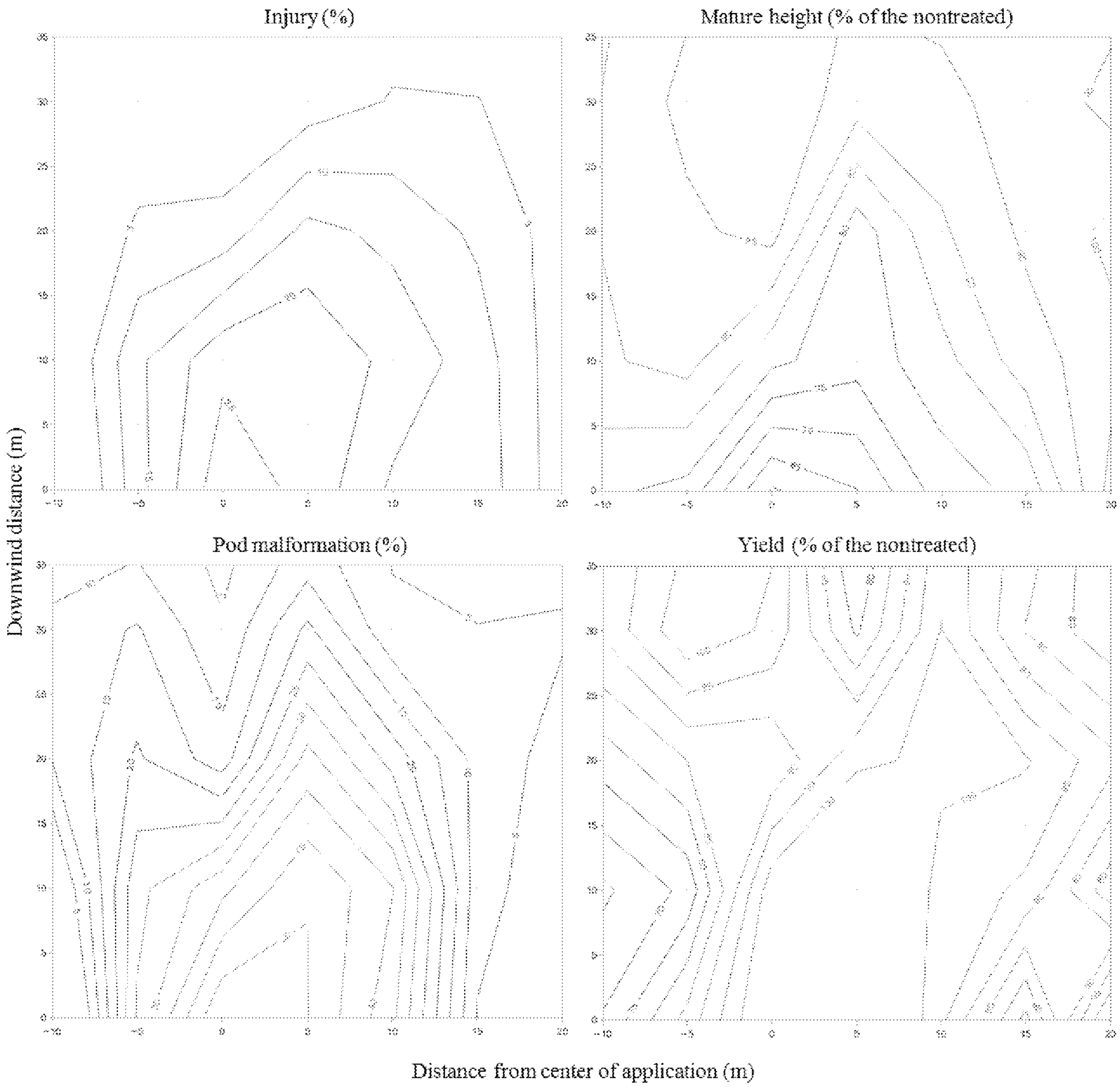
Appendix figure 29. Contour maps illustrating soybean injury, mature height, pod malformation, and yield for trial 4. Soybean injury was rated on a scale from 0 to 100% with 0% being no injury and 100% being plant death. Pod malformation is presented as a percent of total pods malformed. The untreated is the average mature height or yield of 3 random plots within the trial (but outside the drift plume) observed to have no injury at 28 days after application (DAA).



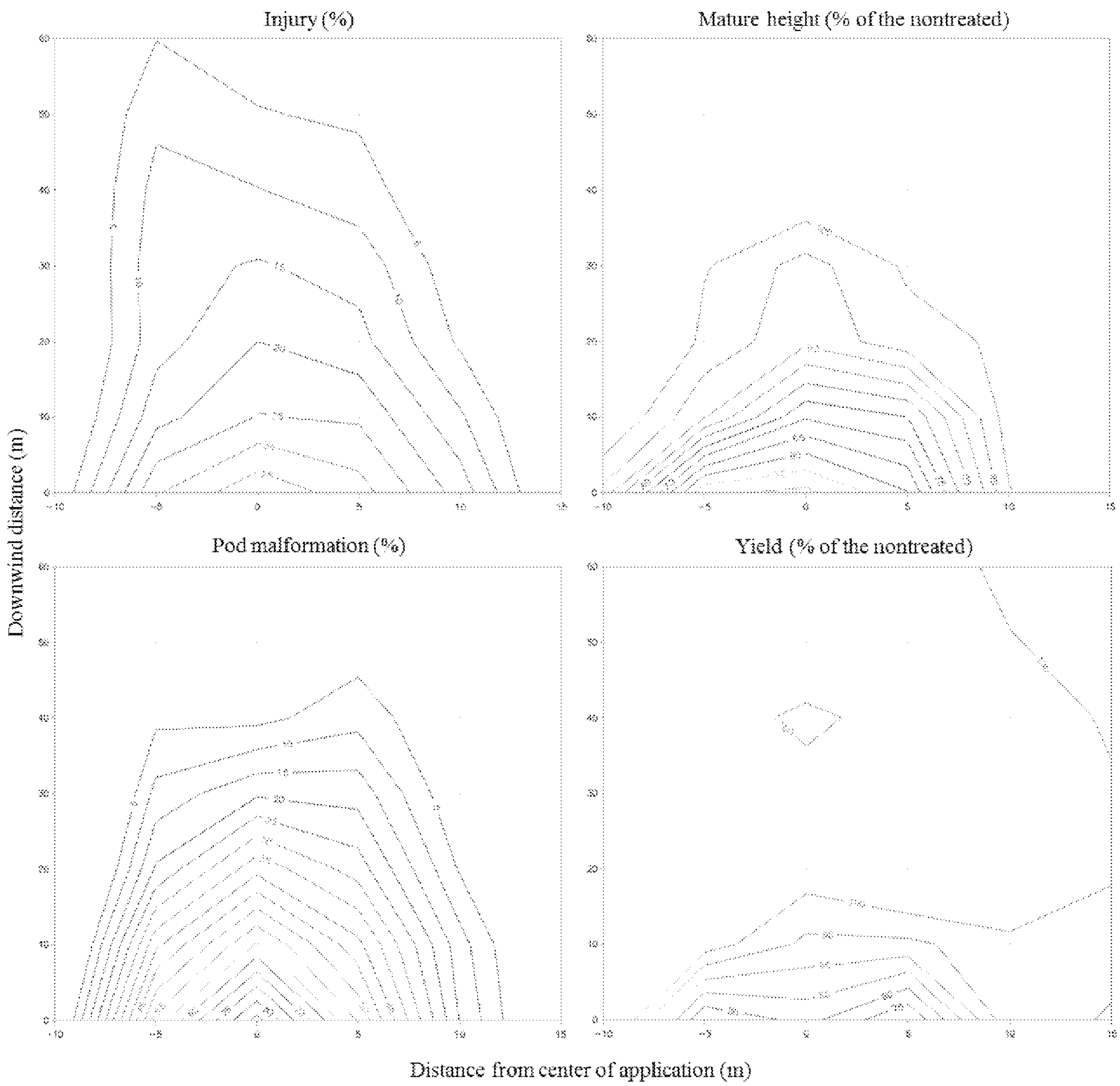
Appendix figure 30. Contour maps illustrating soybean injury, mature height, pod malformation, and yield for trial 5. Soybean injury was rated on a scale from 0 to 100% with 0% being no injury and 100% being plant death. Pod malformation is presented as a percent of total pods malformed. The untreated is the average mature height or yield of 3 random plots within the trial (but outside the drift plume) observed to have no injury at 28 days after application (DAA).



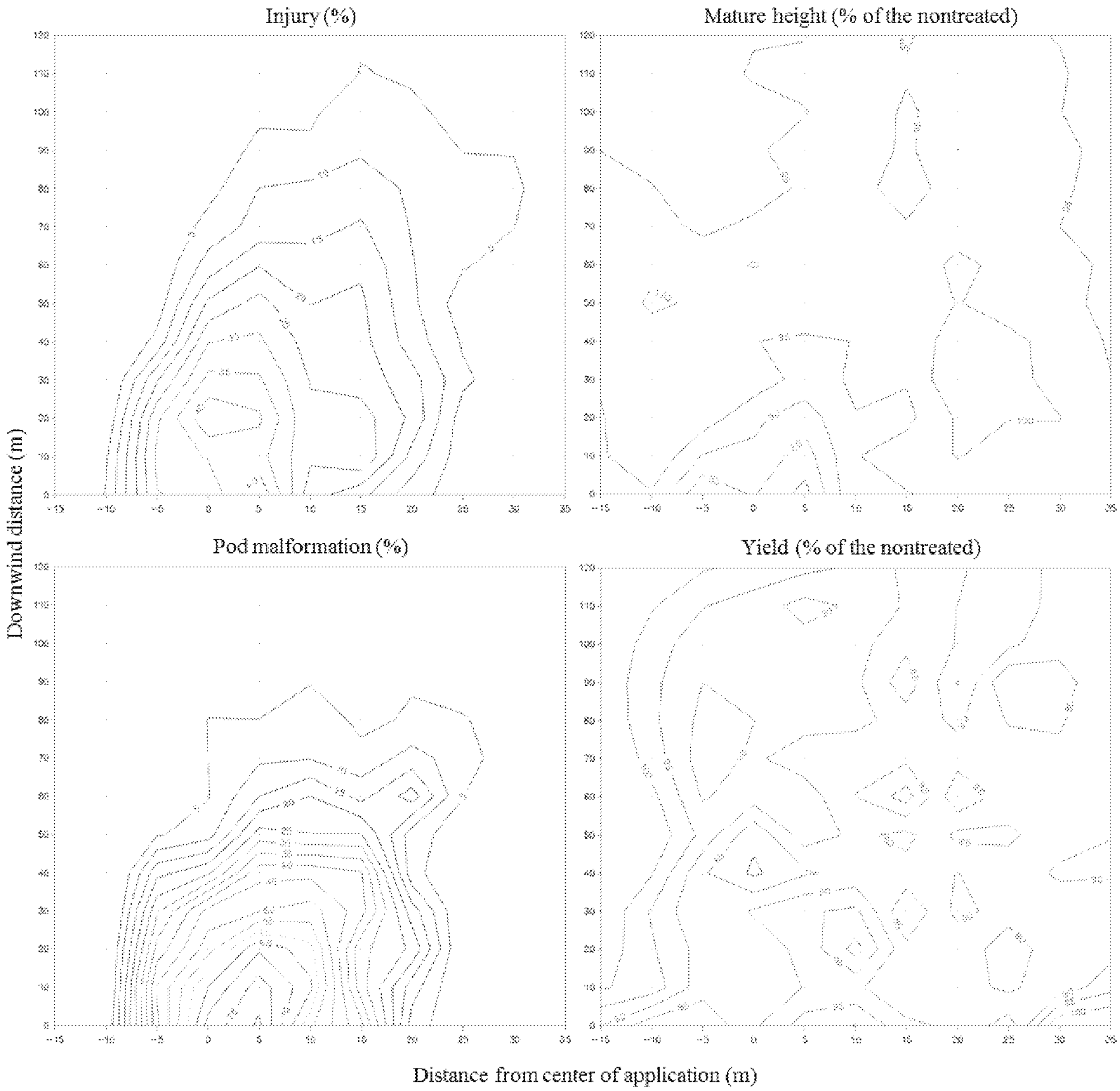
Appendix figure 31. Contour maps illustrating soybean injury, mature height, pod malformation, and yield for trial 6. Soybean injury was rated on a scale from 0 to 100% with 0% being no injury and 100% being plant death. Pod malformation is presented as a percent of total pods malformed. The untreated is the average mature height or yield of 3 random plots within the trial (but outside the drift plume) observed to have no injury at 28 days after application (DAA).



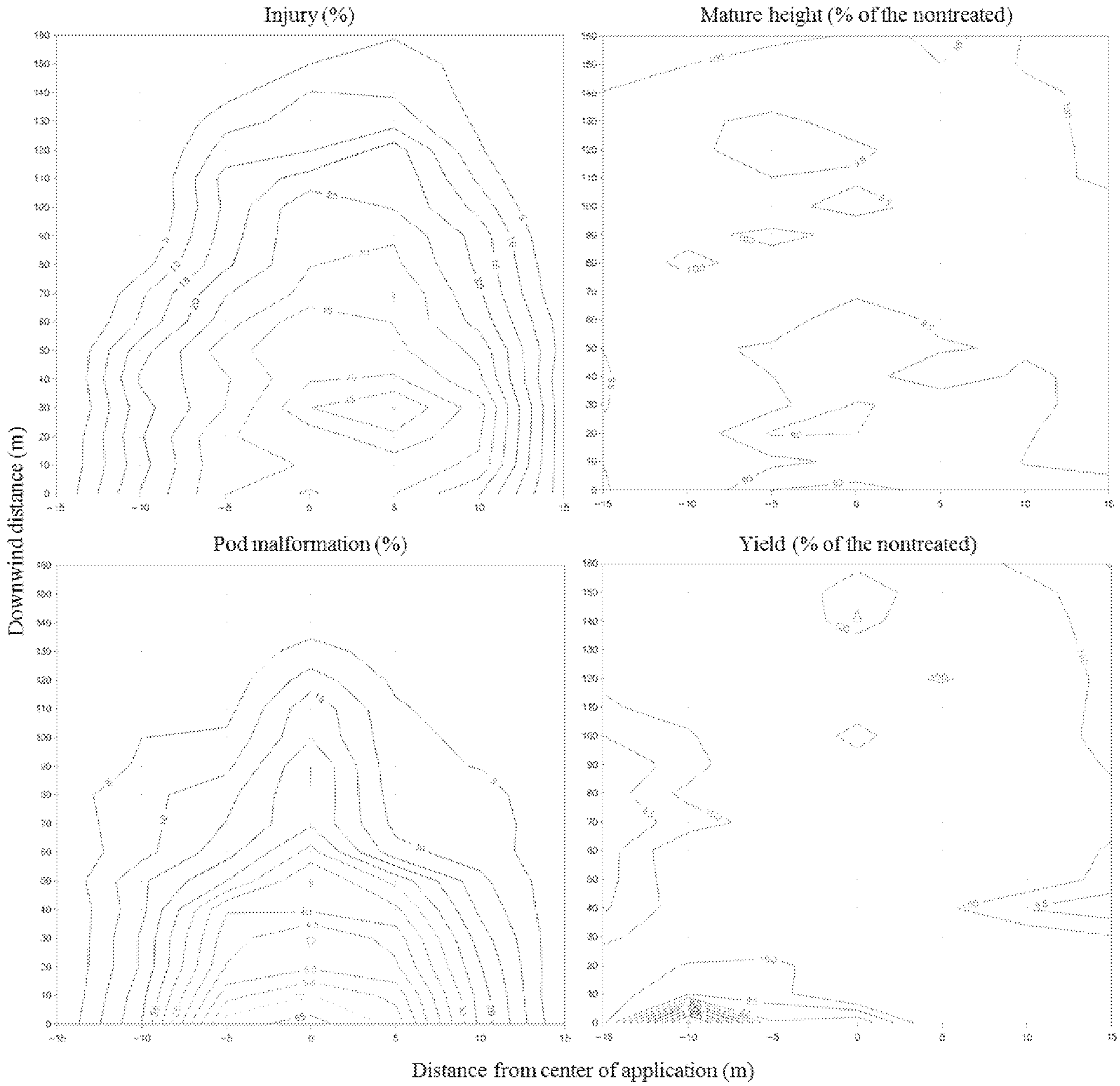
Appendix figure 32. Contour maps illustrating soybean injury, mature height, pod malformation, and yield for trial 7. Soybean injury was rated on a scale from 0 to 100% with 0% being no injury and 100% being plant death. Pod malformation is presented as a percent of total pods malformed. The untreated is the average mature height or yield of 3 random plots within the trial (but outside the drift plume) observed to have no injury at 28 days after application (DAA).



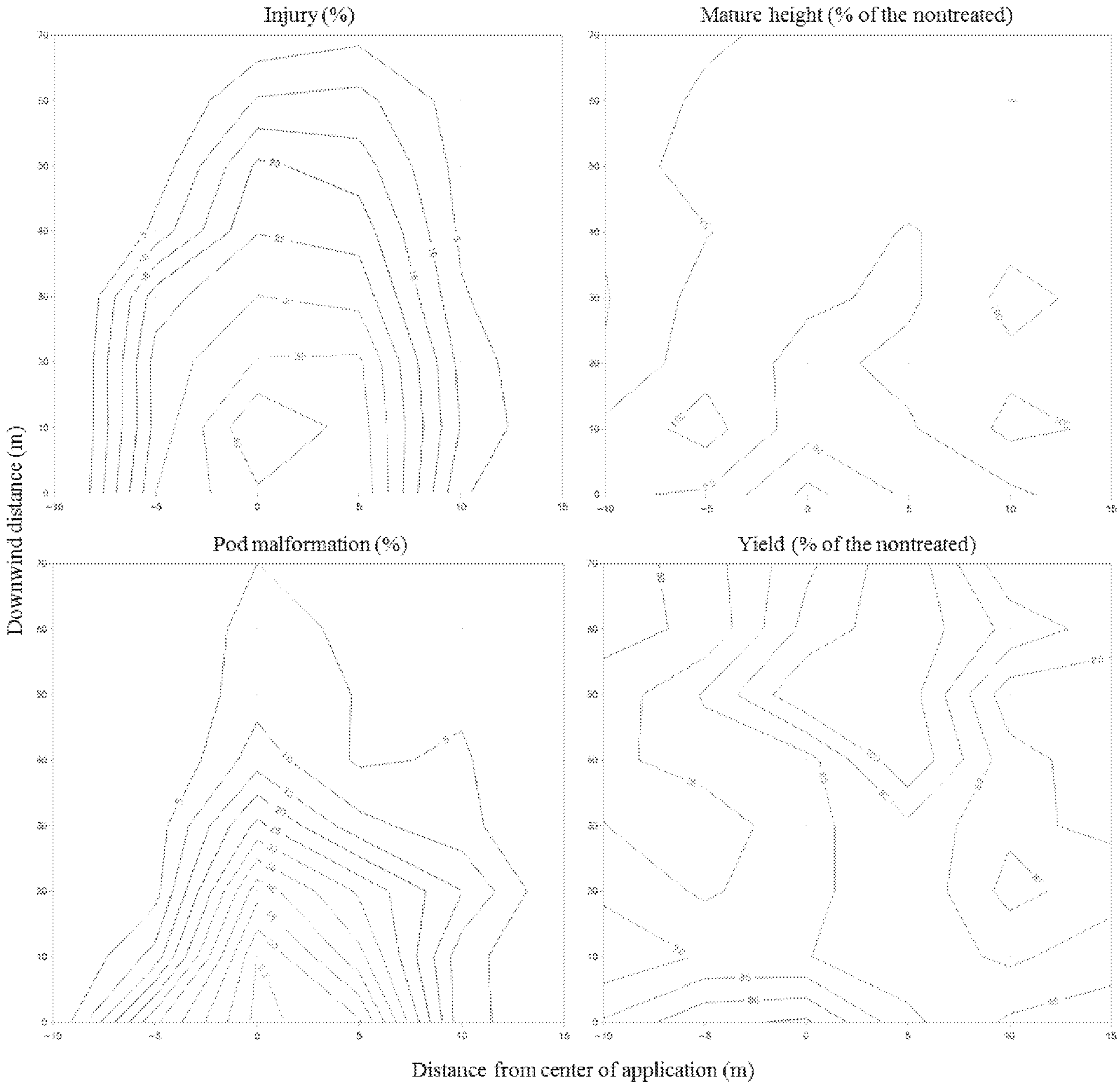
Appendix figure 33. Contour maps illustrating soybean injury, mature height, pod malformation, and yield for trial 8. Soybean injury was rated on a scale from 0 to 100% with 0% being no injury and 100% being plant death. Pod malformation is presented as a percent of total pods malformed. The untreated is the average mature height or yield of 3 random plots within the trial (but outside the drift plume) observed to have no injury at 28 days after application (DAA).



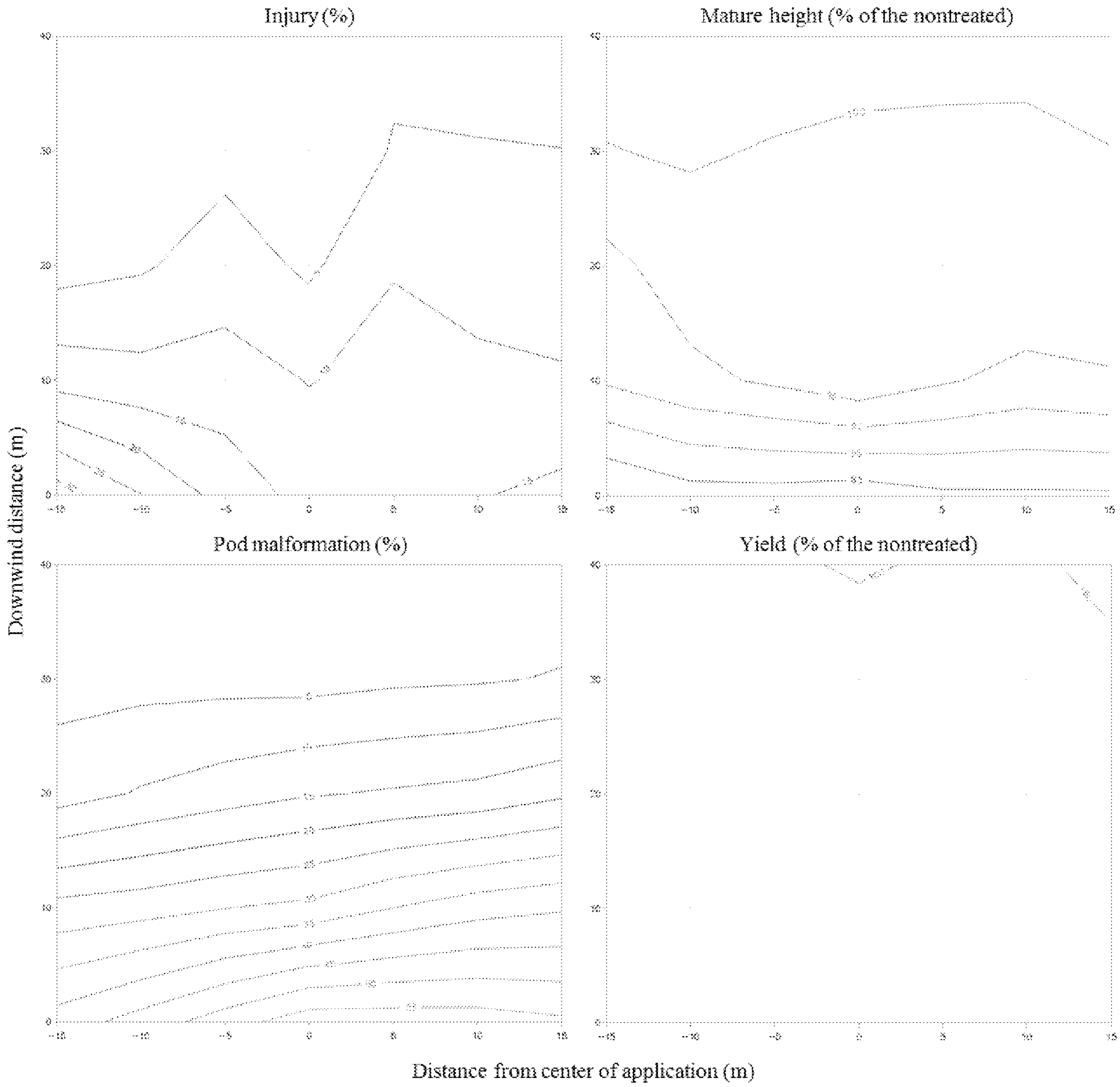
Appendix figure 34. Contour maps illustrating soybean injury, mature height, pod malformation, and yield for trial 9. Soybean injury was rated on a scale from 0 to 100% with 0% being no injury and 100% being plant death. Pod malformation is presented as a percent of total pods malformed. The untreated is the average mature height or yield of 3 random plots within the trial (but outside the drift plume) observed to have no injury at 28 days after application (DAA).



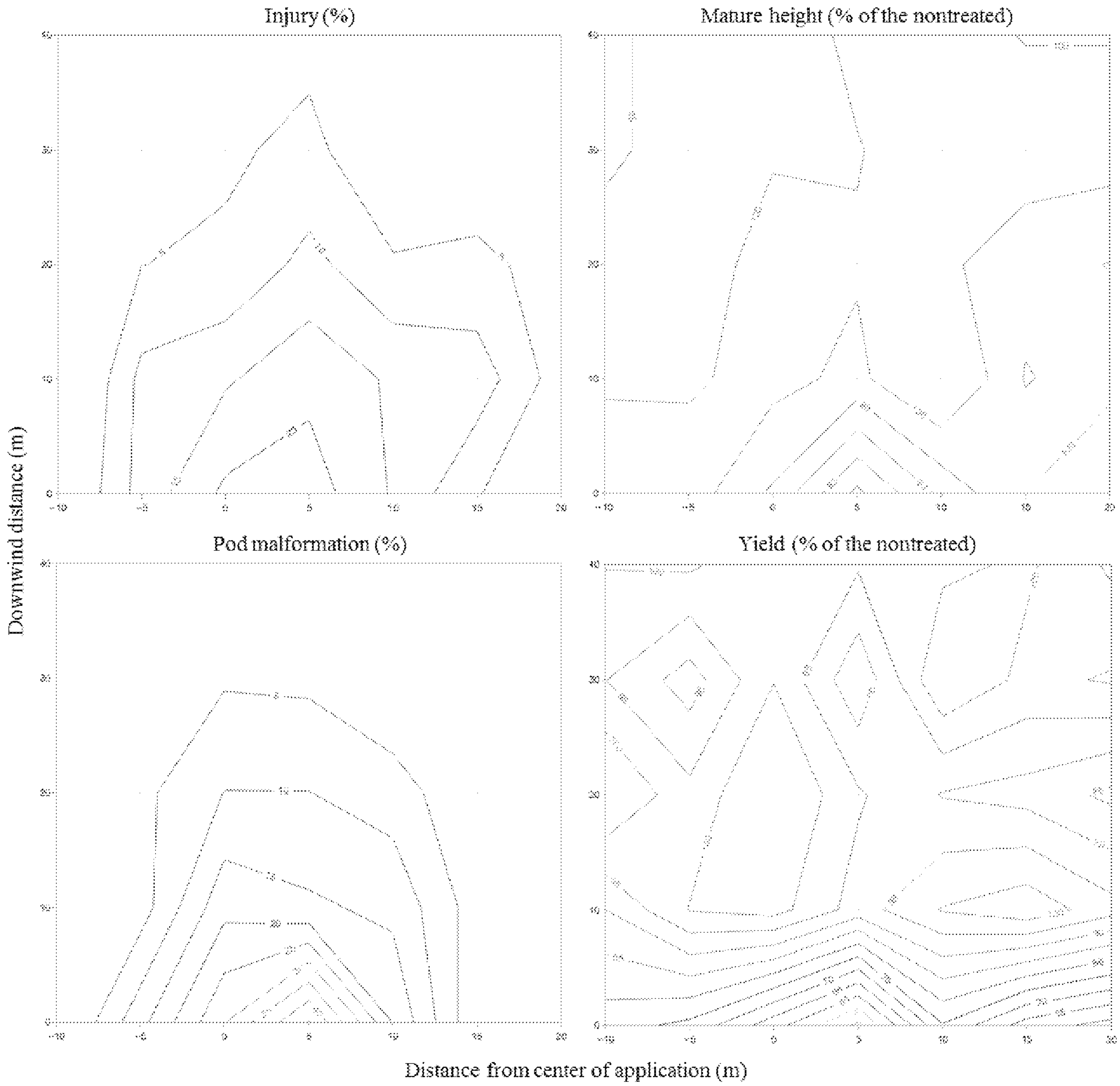
Appendix figure 35. Contour maps illustrating soybean injury, mature height, pod malformation, and yield for trial 10. Soybean injury was rated on a scale from 0 to 100% with 0% being no injury and 100% being plant death. Pod malformation is presented as a percent of total pods malformed. The untreated is the average mature height or yield of 3 random plots within the trial (but outside the drift plume) observed to have no injury at 28 days after application (DAA).



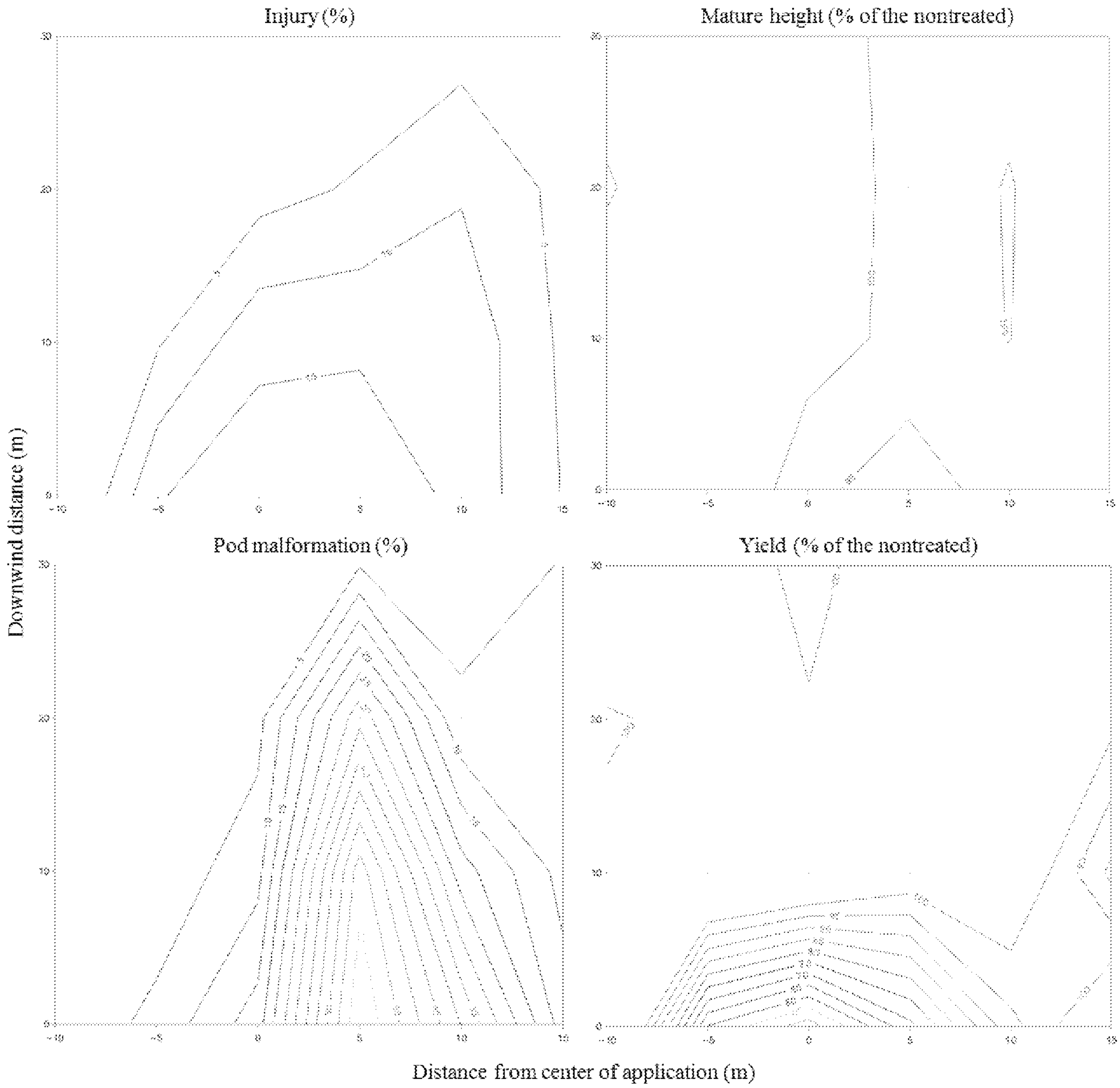
Appendix figure 36. Contour maps illustrating soybean injury, mature height, pod malformation, and yield for trial 11. Soybean injury was rated on a scale from 0 to 100% with 0% being no injury and 100% being plant death. Pod malformation is presented as a percent of total pods malformed. The untreated is the average mature height or yield of 3 random plots within the trial (but outside the drift plume) observed to have no injury at 28 days after application (DAA).



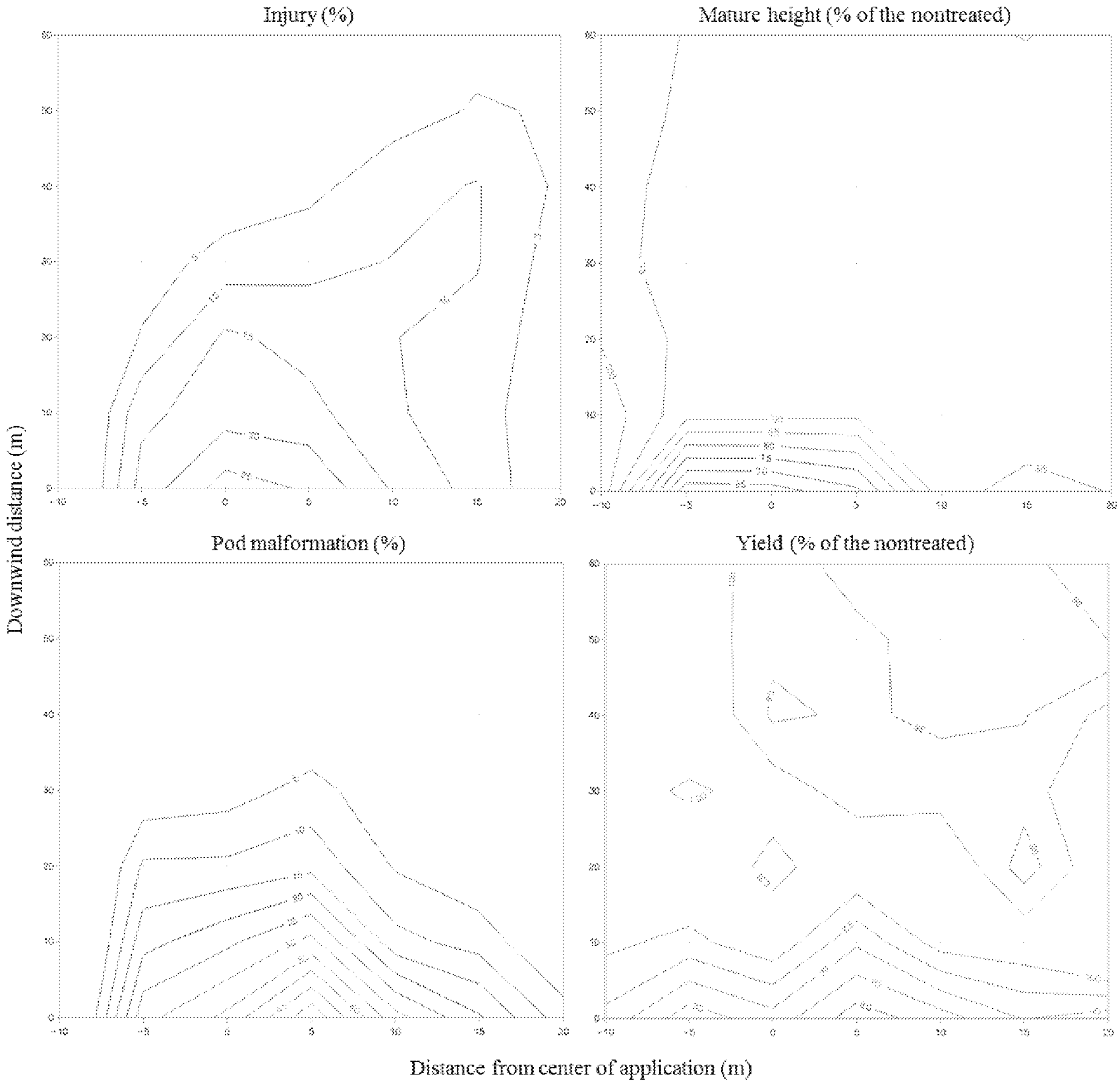
Appendix figure 37. Contour maps illustrating soybean injury, mature height, pod malformation, and yield for trial 12. Soybean injury was rated on a scale from 0 to 100% with 0% being no injury and 100% being plant death. Pod malformation is presented as a percent of total pods malformed. The untreated is the average mature height or yield of 3 random plots within the trial (but outside the drift plume) observed to have no injury at 28 days after application (DAA).



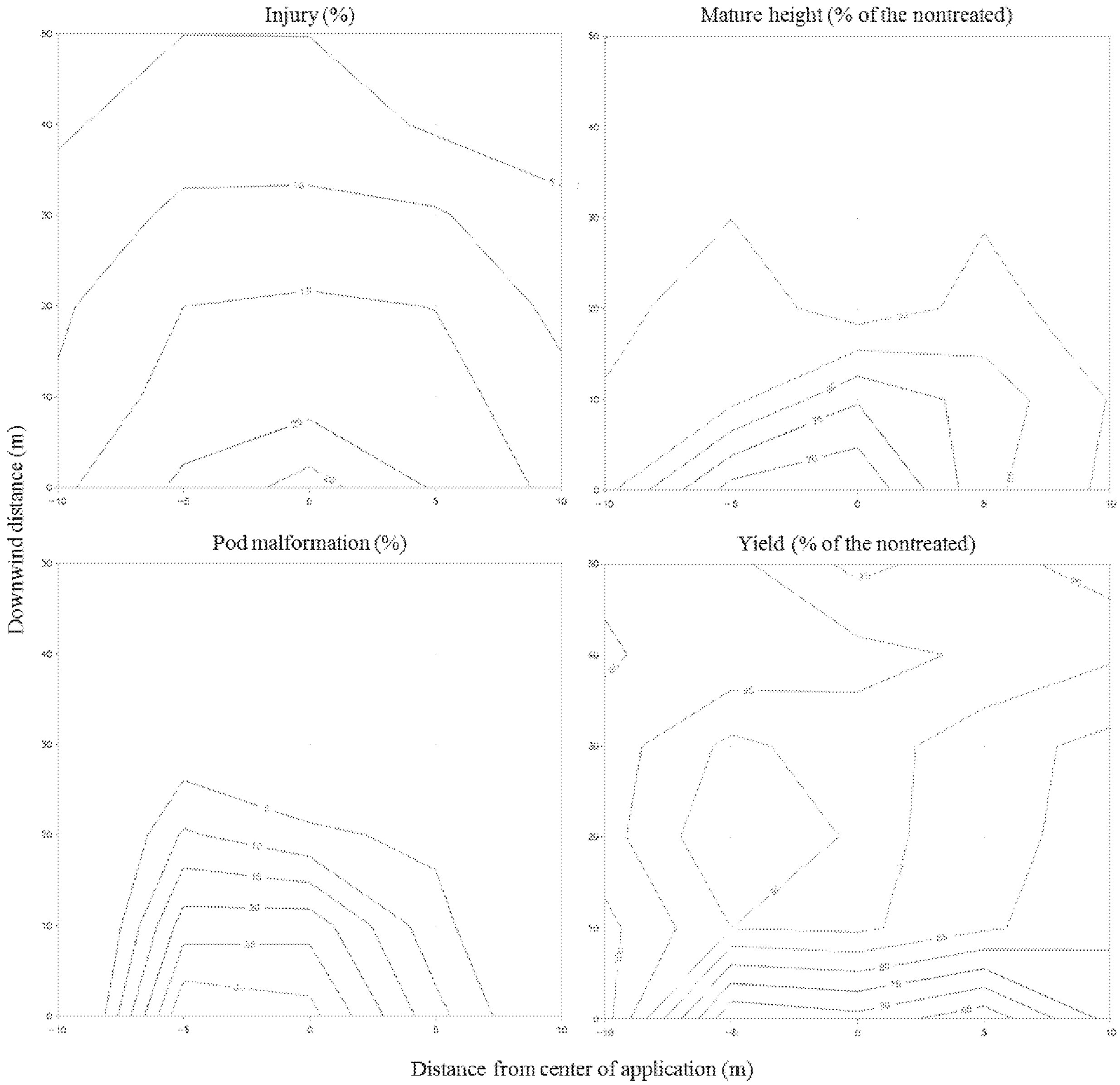
Appendix figure 38. Contour maps illustrating soybean injury, mature height, pod malformation, and yield for trial 13. Soybean injury was rated on a scale from 0 to 100% with 0% being no injury and 100% being plant death. Pod malformation is presented as a percent of total pods malformed. The untreated is the average mature height or yield of 3 random plots within the trial (but outside the drift plume) observed to have no injury at 28 days after application (DAA).



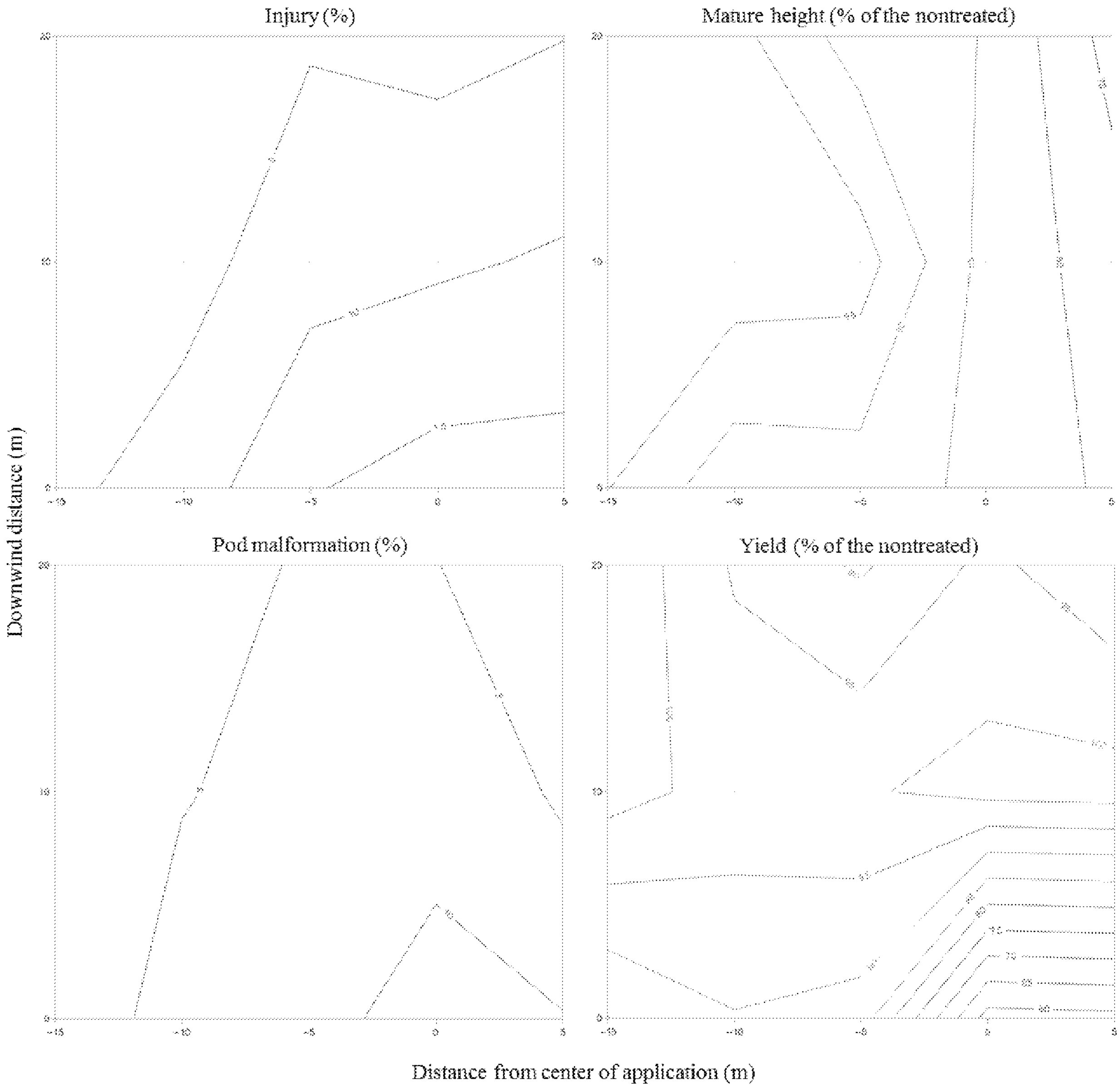
Appendix figure 39. Contour maps illustrating soybean injury, mature height, pod malformation, and yield for trial 14. Soybean injury was rated on a scale from 0 to 100% with 0% being no injury and 100% being plant death. Pod malformation is presented as a percent of total pods malformed. The untreated is the average mature height or yield of 3 random plots within the trial (but outside the drift plume) observed to have no injury at 28 days after application (DAA).



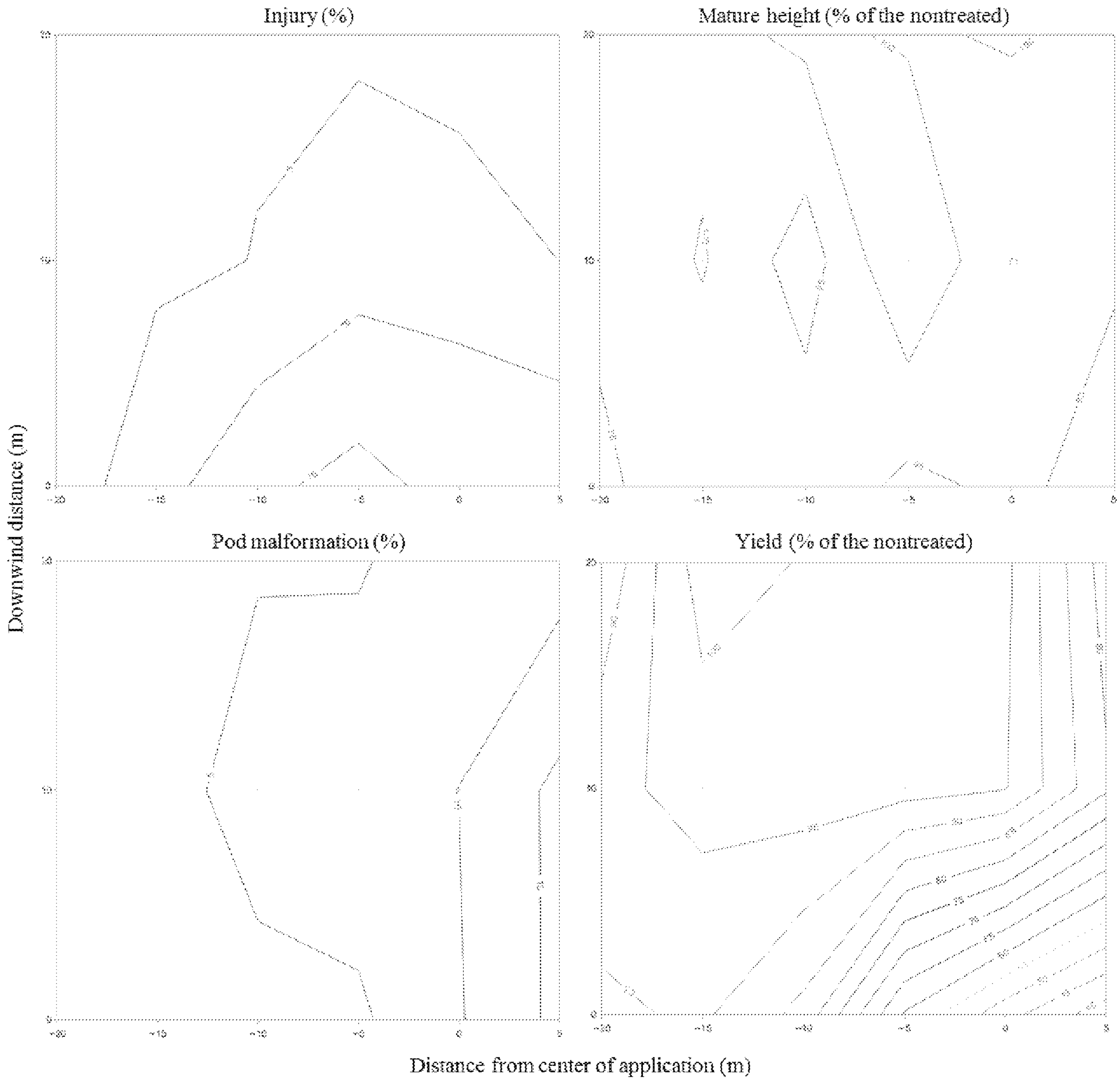
Appendix figure 40. Contour maps illustrating soybean injury, mature height, pod malformation, and yield for trial 15. Soybean injury was rated on a scale from 0 to 100% with 0% being no injury and 100% being plant death. Pod malformation is presented as a percent of total pods malformed. The untreated is the average mature height or yield of 3 random plots within the trial (but outside the drift plume) observed to have no injury at 28 days after application (DAA).



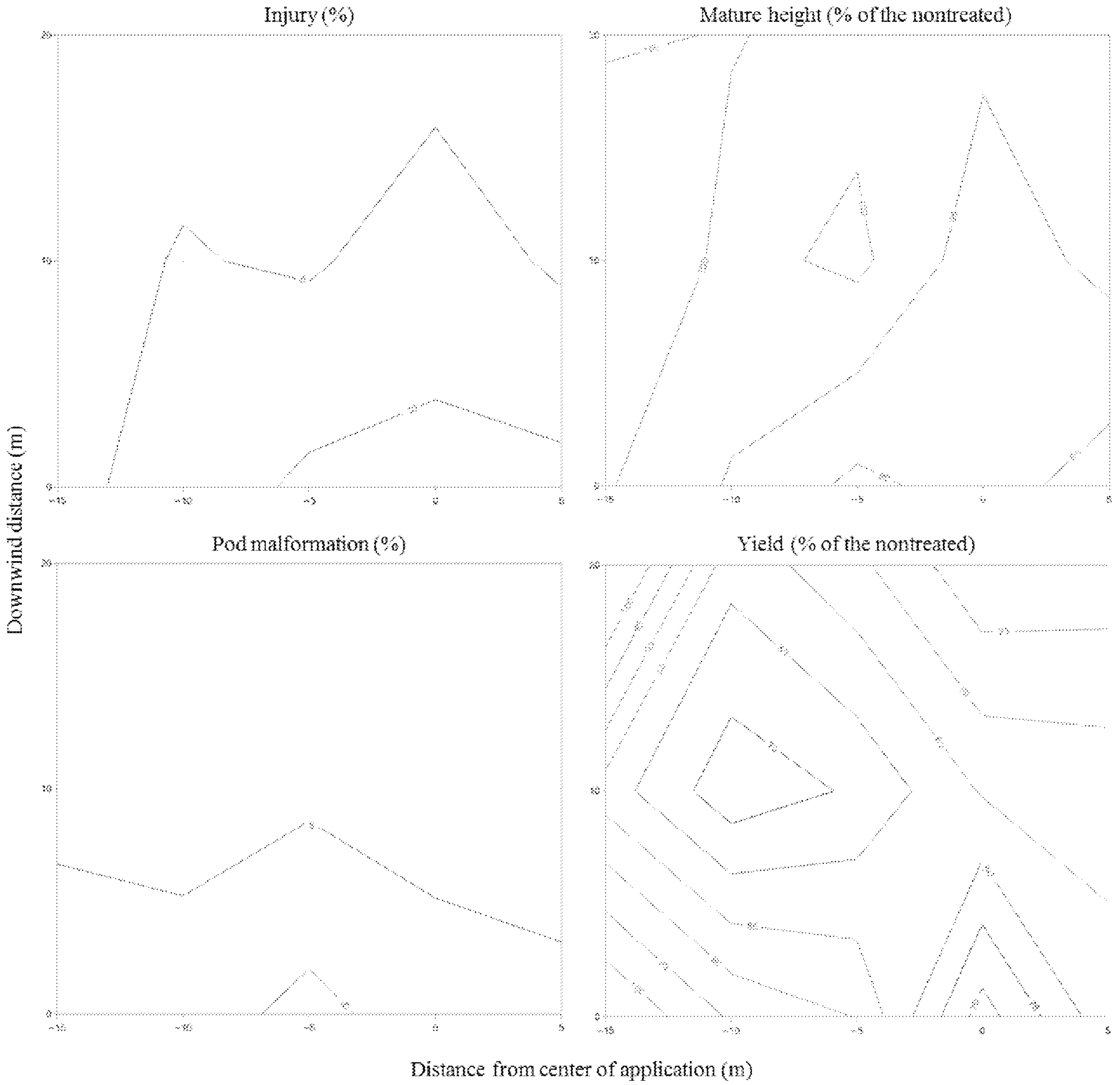
Appendix figure 41. Contour maps illustrating soybean injury, mature height, pod malformation, and yield for trial 16. Soybean injury was rated on a scale from 0 to 100% with 0% being no injury and 100% being plant death. Pod malformation is presented as a percent of total pods malformed. The untreated is the average mature height or yield of 3 random plots within the trial (but outside the drift plume) observed to have no injury at 28 days after application (DAA).



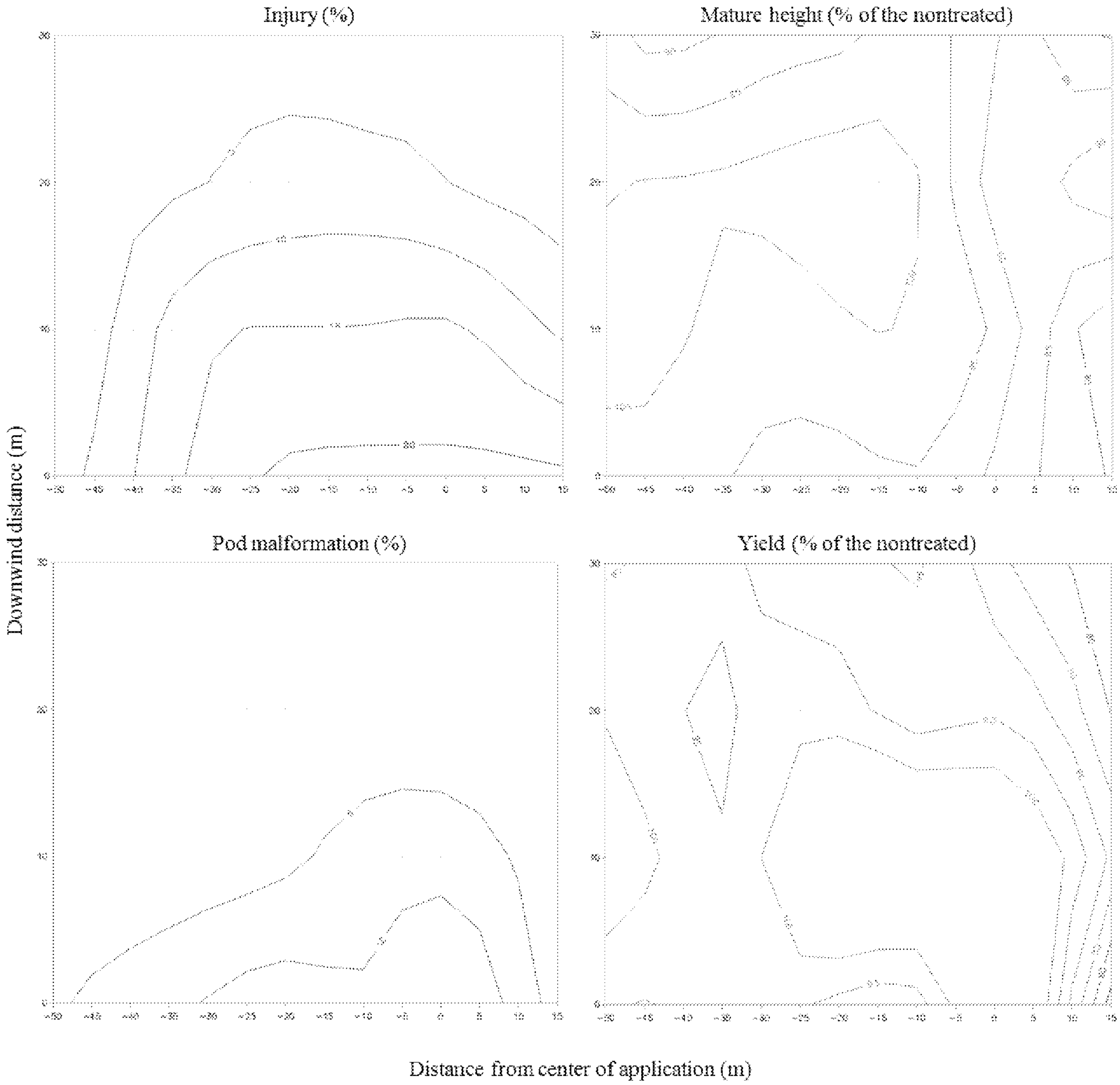
Appendix figure 42. Contour maps illustrating soybean injury, mature height, pod malformation, and yield for trial 17. Soybean injury was rated on a scale from 0 to 100% with 0% being no injury and 100% being plant death. Pod malformation is presented as a percent of total pods malformed. The untreated is the average mature height or yield of 3 random plots within the trial (but outside the drift plume) observed to have no injury at 28 days after application (DAA).



Appendix figure 43. Contour maps illustrating soybean injury, mature height, pod malformation, and yield for trial 18. Soybean injury was rated on a scale from 0 to 100% with 0% being no injury and 100% being plant death. Pod malformation is presented as a percent of total pods malformed. The untreated is the average mature height or yield of 3 random plots within the trial (but outside the drift plume) observed to have no injury at 28 days after application (DAA).



Appendix figure 44. Contour maps illustrating soybean injury, mature height, pod malformation, and yield for trial 19. Soybean injury was rated on a scale from 0 to 100% with 0% being no injury and 100% being plant death. Pod malformation is presented as a percent of total pods malformed. The untreated is the average mature height or yield of 3 random plots within the trial (but outside the drift plume) observed to have no injury at 28 days after application (DAA).



Appendix figure 45. Contour maps illustrating soybean injury, mature height, pod malformation, and yield for trial 20. Soybean injury was rated on a scale from 0 to 100% with 0% being no injury and 100% being plant death. Pod malformation is presented as a percent of total pods malformed. The untreated is the average mature height or yield of 3 random plots within the trial (but outside the drift plume) observed to have no injury at 28 days after application (DAA).

Chapter 2

Response of Soybean Offspring to a Dicamba Drift Event the Previous Year

Abstract

With the advent of dicamba-resistant crops and use of dicamba postemergence to dicamba-resistant soybean and cotton, there will be increased use and thus risk for off-target movement of the herbicide. In the occurrence of dicamba drift, it is not well understood what measurements from soybean plants would correlate with damage to soybean offspring; therefore, possible relationships are of great interest. Sixteen drift trials were established over two years at the Northeast Research and Extension Center in Keiser, AR. A single 8-m-wide by 30- or 60-m-long pass with a high-clearance sprayer was made in each soybean field, resulting in a dicamba drift event. Seeds were collected from plants in each drift trial and planted in a greenhouse in 2015 and 2016. Data were subjected to correlation analysis to determine pairwise associations among parent and offspring observations. Auxin-like symptomology in offspring consistent with dicamba, primarily as leaf cupping, appeared in plots at the unifoliate and first trifoliate stages. Auxin-like symptoms were more prevalent in offspring collected from plants from later reproductive stages as opposed to early reproductive. The highest correlation coefficients occurred when parent plants were treated at R5 growth stage. Parent mature pod malformation was correlated with offspring emergence ($r = -0.37$, $p = 0.0082$), vigor ($r = -0.57$, $p = < 0.0001$), injury ($r = 0.93$, $p = < 0.0001$), and percent of plants malformed ($r = 0.92$, $p = < 0.0001$). This research documents that soybean damaged from dicamba drift during stages of reproduction can negatively affect offspring and that parent pod malformation may be indicative of injury to the offspring. The greatest concern for soybean offspring would be in the occurrence of dicamba drift on seed production fields, causing seed quality to suffer or growers to be alarmed by the

occurrence of auxin-like symptoms on plants soon after emergence. Furthermore, dicamba symptomology occurring in newly emerged soybean could be mistaken as recent drift exposure that may result in dicamba misuse complaints being filed where they are not warranted.

Nomenclature: dicamba; soybean, *Glycine max* (L.) Merr.; cotton, *Gossypium hirsutum* (L.)

Key words: Off-target movement, dicamba-like symptomology, leaf malformation, pod malformation, soybean offspring, soybean growth stages

Introduction

With commercialization of dicamba-resistant (DR) cotton and soybean and labeling of dicamba-containing herbicides for over-the-top use (Anonymous 2016a; Anonymous 2016b), the amount of dicamba applied to U.S. row crops will undoubtedly increase. Approximately 50% of row-crop hectares in Arkansas were planted to soybean in 2015 (United States Department of Agriculture 2016). Therefore, the likelihood of DR cultivars being planted near non-DR soybean is high.

Even with new, lower volatility formulations of dicamba available, primary (physical) drift should still be a concern of growers (Norsworthy et al. 2015). When DR soybean and cotton are planted adjacent to non-DR soybean, applicators must be aware of factors that could contribute to off-target movement since non-DR soybean is highly sensitive to dicamba, and rates as low as 0.08 g ae ha^{-1} (1/7000 X of the 560 g ha^{-1} rate) may cause visible injury symptoms such as leaf crinkling or cupping (Weidenhamer et al. 1989). Correct nozzles, proper boom height, proper spray pressure, and approved mixtures will aid in keeping dicamba from moving off-target via physical drift to susceptible soybean (Anonymous 2017a; Anonymous 2017b; Maybank et al. 1978; Wolf et al. 1992).

The incorporation of DR cultivars into soybean production will increase risk for growers planting non-DR soybean fields. In Arkansas, in-crop labeling of dicamba for DR cultivars will expand current dicamba use for preplant or POST corn (*Zea mays* L.) applications in late February through April and for POST applications on DR soybean and cotton, which likely range from May through August (United States Department of Agriculture 2010). Off-target movement to soybean is less likely to occur from preplant or POST corn applications because fewer soybean hectares have emerged, as soybean planting does not typically begin until late

April in most of Arkansas (United States Department of Agriculture 2016). Likewise, the use rate of dicamba in corn is typically less than that labeled for use in DR cotton and soybean. In addition, March and April temperatures are usually mild, and precipitation is common. Conversely, average temperatures increase in all areas of the US by summer, and precipitation has a tendency to become less frequent. High temperatures have been recognized to increase volatility, and rainfall has been documented to virtually eliminate volatility (Behrens and Lueschen 1979).

As dicamba applications extend into mid-summer, so too does concern for off-target movement to reproductive non-DR soybean. Sensitivity to dicamba differs among soybean growth stages, and yield reduction is highest at early flowering growth stages (Auch and Arnold 1978; Griffin et al. 2013; Solomon and Bradley 2014; Wax et al. 1969). In addition, dicamba exposure to soybean at reproductive stages causes dicamba-like symptomology in its offspring (Thompson and Egli 1973; Wax et al. 1969).

Dicamba is a phloem mobile herbicide (Senseman 2007), meaning that when applied it will inherently move to areas of new growth. Vegetative soybean exposure to dicamba has resulted in greater leaf injury than applications made in later reproductive stages when vegetative growth slows (Kelley et al. 2005; Solomon and Bradley 2014; Weidenhamer et al. 1989). Kelley et al. (2005) reported soybean injury increased from 25 to 37% when dicamba at 0.56 g ha^{-1} was applied at vegetative as opposed to reproductive stages. Solomon and Bradley (2014) documented an 11% decrease in soybean injury when application of dicamba at 0.28 g ha^{-1} was delayed from vegetative stage to R2 growth stage. This is likely due to the increased speed and overall amount of vegetative growth that is occurring at pre-bloom stages. Once reproductive growth begins, vegetative growth in the form of new branches and trifoliate leaves declines as reproductive structures such as flowers and pods begin to form. The amount of dicamba moving to leaves in pre-bloom stages is also likely greater than that of reproductive stages and therefore leads to a greater amount of leaf malformation.

Once in reproductive stages, soybean exposure to dicamba may still result in extensive crop injury, albeit less in the form of leaf malformation and more in reproductive functions. Previous studies have documented as much as 17 and 25% soybean injury from dicamba at 0.28 and 0.56 g ha^{-1}, respectively, applied at the R2 growth stage (Kelley et al. 2005; Solomon and Bradley 2014). The reduction in leaf injury from reproductive exposure compared to vegetative exposure is conveyed in other meristematic regions, such as pods, once reproductive growth begins. Pod malformation can be a result of exposure to dicamba during flowering, with the later developing pods being a possible metabolic sink for dicamba. Pod malformation has been documented as an outcome of exposure to dicamba during flowering (R1 and R2) and early pod forming stages (R3) (Auch and Arnold 1978; Weidenhamer et al. 1989). Percentage of pod injury or percentage of pods showing malformed growth was not previously documented in these studies, only the presence or absence of pod malformation.

The effects of dicamba on soybean have also been documented to extend to the offspring in the form of germination reductions (Auch and Arnold 1979; Thompson and Egli 1973; Wax et al. 1969). Vegetative applications of dicamba at rates ranging from 1 to 56 g ha^{-1} did not result in germination reductions (Auch and Arnold 1979). Germination was relatively unaffected (97%) by dicamba at 8.75 g ha^{-1} when applied to soybean in bloom (Wax et al. 1969). However, application of dicamba at 30 g ha^{-1} during flowering or podfill stages allowed for only 50% germination (Thompson and Egli 1973). Furthermore, germination was reduced by 13 to 46%

from early and late pod formation applications of dicamba at 11 to 56 g ha^{-1} (Auch and Arnold 1979).

In addition to germination reductions, offspring malformation occurs following soybean exposure to dicamba. After application of dicamba at 8.75 to 35 g ha^{-1} to parent plants, offspring developed leaf malformation like that seen after dicamba exposure (Wax et al. 1969). In subsequent research, higher rates of dicamba were used (30 to 560 g ha^{-1}) and the effects were more widespread (Thompson and Egli 1973). Seedlings with dicamba injury were present in all treatments, and severe trifoliate injury appeared in 33 to 100% of offspring.

Soybean exposure to dicamba and subsequent evaluations of offspring have typically been studied after direct applications of low dosages of dicamba to plots rather than using seed from an actual drift event. In addition, past research did not document parameters past the V3 stage of soybean offspring. Therefore, the objective of our research was to examine the season-long effects of an actual dicamba drift event on soybean offspring planted in the field the subsequent season.

Materials and Methods

Field drift experiments were conducted in 2014 and 2015 at the University of Arkansas Northeast Research and Extension Center (NEREC) in Keiser, AR, and offspring experiments were completed at the Arkansas Agriculture Research and Extension Center (AAREC) in Fayetteville, AR, in 2015 and 2016. In 2014, eight dicamba drift experiments were established in commercial production fields at the NEREC with two being treated with dicamba at the R3 growth stage and the remaining six treated at the R1 growth stage of soybean (Table 1). Eight additional dicamba drift experiments were established at the same location in 2015 to obtain data from dicamba application at growth stages R2, R3, R5, and R6. All trials were planted at 31 seed

m^{-1} of row on 97-cm centers. Varieties used are listed in Table 1. A single 8-m-wide by 30- or 60-m- long pass was made with a Bowman Mudmaster (Bowman Manufacturing, Newport, AR) high-clearance sprayer during conditions conducive for a drift event (Figure 1). In the treated area, the diglycolamine (DGA) form of dicamba was applied at 560 g ha^{-1} (Clarity, BASF, Research Triangle Park, NC). A non-ionic surfactant was also included in the spray solution at 0.25% v/v (Induce, Helena Chemical Co, Collierville, TN). The spray boom was equipped with AIXR 11003 nozzles (TeeJet Technologies, Springfield, IL) and calibrated to deliver 94 L ha^{-1} at 275 kPa per the anticipated guidelines for the use of dicamba in DR crops (Anonymous 2013). Each application was made with a 60-cm boom height above the soybean canopy while traveling at 15 km h^{-1}. The treated area was 30 m in length for applications when wind directions were less than 45 degrees from the sprayer traveling direction. The field was grid sampled into four rows (spaced 97 cm apart) by 6-m-long plots extending from the application area until no injury was observed at 14 and 28 days after application (DAA). Applications occurring when the wind direction was greater than 45 degrees from the application direction were 60 m in length. Transects were established at 15, 30, and 45 m along the application area that extended perpendicular to the rows. Four-row by 10-m plots were established along each transect until no injury was observed at 14 and 28 DAA. Regardless of wind direction, only the center two rows of each plot within each transect were used for data collection.

Measurements on the parent plants included visual estimates of leaf malformation on a 0 to 100% scale, with 100% being plant death, at 14 and 28 DAA, soybean height at 28 DAA and maturity, percentage of malformed pods at maturity, and grain yield adjusted to 13% moisture. Height and yield measurements were later converted to percentages of the nontreated check plots. Five plots from each trial that were documented to have no leaf malformation at 28 DAA were used to calculate the nontreated check averages for height and yield. A sample (approximately 1 kg) of seed was taken from each plot after harvest and placed in a freezer maintained at -10 C until the following spring when planting occurred.

Seed collected from the 2014 and 2015 drift trials were planted at AAREC in 2015 and 2016, respectively, at 25 seed m^{-1} row in 6-m-long plots on a 91-cm spacing. The site consisted of a Captina silt loam (Fine-silty, siliceous, active, mesic Typic Fragiudults) with a pH of 6.1 and 1.18% organic matter. The field was furrow irrigated weekly if at least a 2.5-cm rainfall did not occur. Initial planting in 2015 was April 26; however, injury in the form of stand loss was caused by preemergence (PRE)-applied flumioxazin (Valor SX, Valent Corporation, Walnut Creek, CA), after which the test was replanted in a different field on June 25. No PRE herbicides were used thereafter to avoid herbicide injury. In 2016, initial planting occurred on May 19. Stand loss occurred due to soil crusting and pigeon (*Columba livia*) feeding in isolated areas of the field to the extent that the experiment was replanted June 9. All varieties were glufosinate-resistant for ease of weed control (Table 1). Multiple varieties were used but all were indeterminate growth habit to reduce variability in response. Currently there is no available research documenting differences in dicamba sensitivity of soybean within growth habit. Experiments were kept weed-free with a POST application of glufosinate (Bayer CropScience, Research Triangle Park, NC) at 595 g ai ha^{-1} and *S*-metolachlor (Syngenta Corporation, Greensboro, NC) at 1,390 g ai ha^{-1} at 21 days after planting (DAP) followed by a second application of glufosinate two weeks later.

Measurements from the offspring included emergence (% of planted seed emerged), vigor (1 to 5), injury at 21 DAP (% visible injury on a 0 to 100% scale with 100% being plant death), number of plants malformed per plot (converted to % of plants showing malformation), and grain yield adjusted to 13% moisture (kg ha^{-1}). Soybean vigor was rated on a scale of 1 to 5 for each plot using the following criteria: 1 = extremely low vigor (slow initial growth with delayed emergence or reduced emergence of >60% under field conditions), 2 = poor vigor (slow initial growth and 30 to 60% reduction in emergence in the field), 3 = moderately low vigor (average initial growth with slight reduction in emergence likely under good field conditions), 4 = moderately high vigor (average initial growth with slight reduction in emergence likely in fields having suboptimal conditions), 5 = extremely high vigor (seedlings quickly emerge; exhibit rapid growth; likely to emergence under a wide array of field conditions). Although a standardized definition of vigor satisfactory to most investigators has yet to be realized, the concept of vigor and its importance in crop development are well accepted (Pollock and Roos 1972). Yield was later converted to percentages relative to the nontreated plots. Five plots from each trial that were documented to have no parent leaf malformation at 28 DAA the previous year were used to calculate the nontreated treatment averages for offspring yield. Data were subjected to correlation analysis using JMP 12 PRO (SAS Institute, Cary, NC) to determine Pearson pairwise correlations among parent and offspring observations.

Results and Discussion

R1 Drift Events. Previous research found soybean exposure to dicamba in early reproductive stages to be detrimental to grain yield (Auch and Arnold 1978; Wax et al. 1969). However, drift events occurring at R1 growth stage resulted in only one significant correlation between parent and offspring variables. Relative mature height of the parent was significantly correlated with offspring injury (r = -0.13) (Table 2; Figure 2). Terminal node inhibition can occur to soybean exposed to dicamba drift or tank contamination. Events that lead to terminal node inhibition will likely result in height reduction at maturity. Solomon and Bradley (2014) documented yield loss to coincide with height reduction caused by dicamba concentrations as low at 2.8 g ha^{-1} (1/400th

of the labeled use rate in soybean) applied at early reproductive soybean stages. Height reduction may be the greatest predictor of yield of soybean directly exposed to dicamba, likely because plants experiencing terminal inhibition received the greatest concentration of dicamba. This may be of significance for soybean offspring. Soybean plants exposed to a drift event may have ample time to detoxify lower concentrations of dicamba; however, higher concentrations may remain active in the plant through seed fill and therefore transported to the seed.

R2 Drift Events. A delay in drift events until R2 provided nine significant linear correlations between parent and offspring variables (Table 2). Soybean parent leaf malformation at 28 DAA was significantly correlated with offspring injury ($r = 0.46$; $p = < 0.0001$) and percent of offspring plants malformed ($r = 0.47$, $p = < 0.0001$). Scatterplots visually document that increased parent leaf malformation leads to an increased risk for offspring injury and percent of plants malformed (Figure 3). Although previous research has documented that visible estimates of injury from dicamba may be a poor indicator and overestimate yield loss (Egan et al. 2014), these data reveal that increased leaf malformation to parent plants after exposure at R2 is a somewhat reliable indicator in the likelihood of dicamba-like symptomology rematerializing in the subsequent offspring.

Parent height at 28 DAA and at maturity following an R2 dicamba drift event was correlated negatively with offspring injury (Table 2). Percent of offspring plants malformed increased with a decrease in parent height at 28 DAA ($r = -0.18$, $p = 0.0011$) and maturity ($r = -0.39$, $p = < 0.0001$). As with R1 applications, it appears that parent height at maturity is a better indicator of possible effects on soybean offspring than height at 28 DAA. Soybean plants experience a decreased rate of vegetative growth as flowers begin to become an energy sink, and therefore, the effect on height reduction may not be realized until plants achieve maximum height.

Perhaps the most intriguing and strongest correlation at this R2 growth stage existed between percentage of parent pods malformed and the offspring variables injury ($r = 0.59$, $p = < 0.0001$) and percentage of plants injured ($r = 0.58$, $p = < 0.0001$) (Table 3). These findings document that prior to pod forming stages, a dicamba drift event may still result in an excessive number of pod malformation on offspring. Dicamba drift onto R2 soybean resulted in up to 75% of pods being malformed nearest the source of the drift (data not shown). As a soybean plant is exposed to increasing amounts of dicamba, pod malformation may increase at this stage because more dicamba will remain active in the plant through pod forming stages. It is thought that non-metabolized dicamba present in the plant after pod formation will likely be transported to the seed during seed filling stages (Thompson and Egli 1973). Thus, high numbers of malformed pods resulting from an R2 drift event can result in injury to offspring.

R3 Drift Events. Thompson and Egli (1973) documented offspring trifoliate injury to increase two-fold when low doses of dicamba were applied to parent plants during pod forming stages compared to flowering. With an actual dicamba drift event, maximum percentage of offspring injured increased from 11% after R1 events to 50% from R3 drift events (data not shown). Therefore, with delayed drift exposure, soybean has less time to metabolize dicamba prior to it being moved to the sink once seed fill begins. Exposure of soybean to radiolabeled dicamba at different reproductive growth stages and assessing the metabolites is one way to test this hypothesis.

Percentage of malformed parent pods displayed the highest correlation coefficients for offspring vigor, injury, and percentage of plants injured (Table 2; Figure 4). As with R2 drift events, parent plants exposed to R3 drift events displayed extensive pod malformation, which ranged from 0 to 70% depending on distance from the drift event (data not shown). The vast range of pod malformation aided in picking up correlations among offspring variables when even slight changes in injury and vigor were noticed. Based on these data, the amount of pod malformation seen after an R3 drift event could be used to assess the likelihood of soybean offspring having reduced vigor and dicamba-like symptoms.

R5 Drift Events. Drift events at R5 resulted in a significant correlation between parent pod malformation and offspring emergence ($r = -0.37$, $p = 0.0082$), which was the only occurrence of a relationship with offspring emergence in these experiments (Table 2; Figure 5). It may be that the presence of dicamba at the beginning of seed formation allowed for more dicamba to be moved to the seed, resulting in a concentration high enough to reduce emergence. In other research, soybean exposure to a sub-lethal dose of dicamba at the R5 growth stage was shown to reduce germination of the offspring (Barber et al. 2015).

Percentage pod malformation of parent plants was involved with more and higher correlations than any other parent variable. However, percentage of parent pods malformed ranged from only 0 to 15%, which likely led to the steeper correlations (data not shown). The decrease in pod malformation from 75 and 70% maximums at R2 and R3 to 15% at R5 (data not shown) can be explained by the focus of plant growth at the time of application. At R2, plants have yet to start pod formation, and R3 marks only the presence of a 0.5-cm pod on the upper four nodes, whereas R5 denotes the completion of pod formation and the beginning of seed growth (although the plant continues to flower and produce pods/seeds near the terminals). Dicamba remaining in the soybean plant after R2 and R3 exposure has the capacity to disrupt pod formation to a much greater extent than exposure at R5, as pod formation has concluded by

R5. However, pod malformation was still seen after R5 exposure due to the indeterminate growth habit of the soybean variety. Malformed pods were seen only in the uppermost nodes that were still showing growth. Furthermore, with the drift event occurring after most pods were formed, dicamba could rapidly move to the seed. Thus, an increase in the number of malformed offspring would be expected. In fact, the maximum amount of plants injured per plot increased from 50% after R3 drift events to 99% after R5 drift events (data not shown). Therefore, after an actual dicamba drift event at R5 growth stage, high numbers of malformed parent pods may indicate the likelihood for more offspring plants to display abnormal growth and a higher percentage of offspring injury as well as a possible decrease in offspring vigor and emergence.

R6 Drift Events. Parent injury and canopy heights 28 DAA could not be recorded after R6 drift events as leaf drop had started to occur approximately 2 weeks after application and plants were mature in most cases at 28 DAA. Lack of growth after initiation of drift events to R6 soybean likely led to the absence of significant correlations with parent pod malformation and mature height. Furthermore, since injury was not obvious, plots only extended 18 to 24 m from the drift event. Parent mature height was reduced by a maximum of only 11% after R6 application, whereas earlier applications reduced mature height by as much as 61% (data not shown). Parent pod malformation was nearly nonexistent and only ranged from 0 to 1% (Figure 6).

Relative yield of offspring was reduced by as much as 42% at R6 and was the only parent variable to be correlated with offspring variables. As the relative yield of parent plants decreased, so did offspring vigor ($r = 0.41$, $p = 0.0028$) (Table 2). Reductions in offspring injury ($r = -0.43$, $p = 0.0016$) and percent of plants injured ($r = -0.49$, $p = 0.0028$) were documented when parent relative yield was increased (Figure 6). Yield reduction may occur for a multitude of reasons, and this research documents that dicamba exposure to soybean at R6 may not be identifiable due to lack of leaf or pod malformation. For these reasons, dicamba exposure to soybean at R6 may be most worrisome to the seed production industry. General germination tests may not identify dicamba exposure because offspring of soybean exposed to drift events at R6 did not have a noticeable reduction in emergence. If dicamba exposure is suspected, soybean offspring may need to be grown to the V2 or V3 stages to examine if leaf malformation will appear.

Practical Implications. It is possible that the replanting of this study later in summer may have resulted in better growing conditions than those early in the spring; therefore, an even greater difference in vigor may result under less than ideal growing conditions following planting. Yield loss is perhaps the most important variable for most growers. The replanting of these trials coincided more with a double-crop planting date, likely resulting in reduced yield from full-season planting dates. Typically, double-crop soybean is planted in narrow rows to maximize yield as reduced vegetative growth will occur when compared to full-season soybean (Harder et al. 2007; Johnson et al. 2002). It is likely that a decrease in row spacing would have increased the capacity to yield by increasing leaf area index and shortening the amount of time until soybean canopy formation (Harder et al. 2007). Further research is needed to examine the relationship between offspring yield after parent exposure to dicamba.

The potential to have dicamba applied near fields of soybean that are already in reproductive stages is high in the midsouthern USA. In Arkansas, soybean has a wide window of planting time that ranges from April through July (NASS 2010). Therefore, early-planted soybean could be in close proximity to late-planted double-crop soybean. Applications of dicamba to DR double-crop soybean would take place at a time when neighboring early-season soybean will be in reproductive stages. It is well known that soybean is highly sensitive to dicamba, and this research documents that effects may be transmitted to offspring from actual drift events at reproductive stages.

One instance of concern is dicamba drift onto seed production fields. Dicamba symptomology was not readily visible when actual drift events occurred at seed filling stages. For example, there was an overall reduction in parent leaf malformation caused by dicamba drift with progression of soybean maturity as seen in Figures 2 to 6. Therefore, exposure to dicamba may not be realized without close inspection of fields during reproductive development. Subsequent germination tests may pick up seed exposed to higher rates as documented in previous research (Auch and Arnold 1978; Thompson and Egli 1973). However, these actual drift events only produced one significant relationship with offspring emergence, which occurred with parent pod malformation at R5 timing. Therefore, it is possible for contaminated seed to germinate normally, yet still display auxin-like symptomology after germination. Thus, seeds that have been unknowingly exposed to a dicamba drift event may be distributed to growers, and after emergence, plants may display dicamba-like symptoms and cause growers to place blame on others.

Although there is a need for DR technology to provide diversity in soybean weed control programs and to manage resistant weeds, the risk for damage to neighboring soybean fields and contamination of seed production fields should be weighed. Previous studies have documented the dangers of dicamba to soybean seed production on a small scale with direct application; however, these experiments document that those effects can also be seen after actual dicamba drift events and extreme caution is needed when applying dicamba in the vicinity of non-DR soybean.

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Table 1. Year, trial, soybean variety, growth stage, and number of observations in parent drift trials at the Northeast Research and Extension Center in Keiser, AR.

Year	Trial	Variety	Growth stage	Observations
2014	14-1	Progeny 4819	R1	88
2014	14-2	Halo 494	R1	84
2014	14-3	Halo 494	R1	76
2014	14-4	Halo 494	R1	104
2014	14-5	HBK 4850	R1	54
2014	14-6	HBK 4850	R1	65
2014	14-7	Progeny 4819	R3	65
2014	14-8	Progeny 4819	R3	57
2015	15-1	Delta Grow 4767	R3	63
2015	15-2	Delta Grow 4767	R3	50
2015	15-3	Credenz 4950	R2	188
2015	15-4	Credenz 4950	R2	132
2015	15-5	Progeny 4814	R5	52
2015	15-6	Credenz 4950	R6	15
2015	15-7	Credenz 4950	R6	15
2015	15-8	Progeny 4814	R6	21

Table 2. Pearson's correlation coefficients between parent and offspring variables at each respective growth stage.[ab]

Parent variables	Growth stage	Offspring variables: Emergence (%)	Vigor	Injury (%)	% of plants injured	Relative yield (%)
Leaf malformation at 28 DAA (%)	R1	-0.04	-0.02	0.06	0.01	0.10
	R2	-0.08	-0.12	0.46*	0.46*	0.12
	R3	-0.02	-0.02	0.45*	0.31*	-0.17
	R5	-0.23	-0.41*	0.74*	0.72*	-0.22
	R6	-	-	-	-	-
Height at 28 DAA[c] (% of check)	R1	0.03	0.09	-0.05	0.00	-0.12
	R2	-0.05	0.02	-0.21*	-0.18*	-0.10
	R3	-0.01	0.00	-0.31*	-0.16	0.07
	R5	-0.27	-0.26	0.39*	0.38*	-0.08
	R6	-	-	-	-	-
Height at maturity (% of check)	R1	-0.01	0.01	-0.13*	-0.02	-0.10
	R2	0.11	0.10	-0.37*	-0.39*	0.00
	R3	0.06	0.00	-0.21*	-0.06	0.19*
	R5	0.11	0.05	-0.09	-0.09	-0.09
	R6	0.18	0.18	-0.23	-0.21	0.31
Pods malformed (% of total)	R1	-0.07	-0.07	0.10	0.01	0.07
	R2	-0.06	-0.09	0.59*	0.58*	-0.02
	R3	-0.15	-0.21*	0.51*	0.41*	-0.04
	R5	-0.37*	-0.57*	0.93*	0.92*	-0.34
	R6	0.18	-0.35	0.33	0.32	0.03

Table 2 continued

		Offspring variables				
Parent variables	Growth stage	Emergence (%)	Vigor	Injury (%)	% of plants injured	Relative yield (%)
Relative yield (%)	R1	-0.02	0.08	-0.09	-0.01	-0.01
	R2	-0.15	-0.12	-0.03	-0.01	0.30*
	R3	0.04	0.11	-0.39*	-0.26*	0.05
	R5	0.01	-0.09	0.13	0.13	0.02
	R6	0.15	0.41*	-0.43*	-0.49*	0.09

[a]*Indicates significance to $\alpha = 0.01$

[b]Sample sizes: R1(471), R2(320), R3(235), R5(52), R6(51)

[c]Days after application

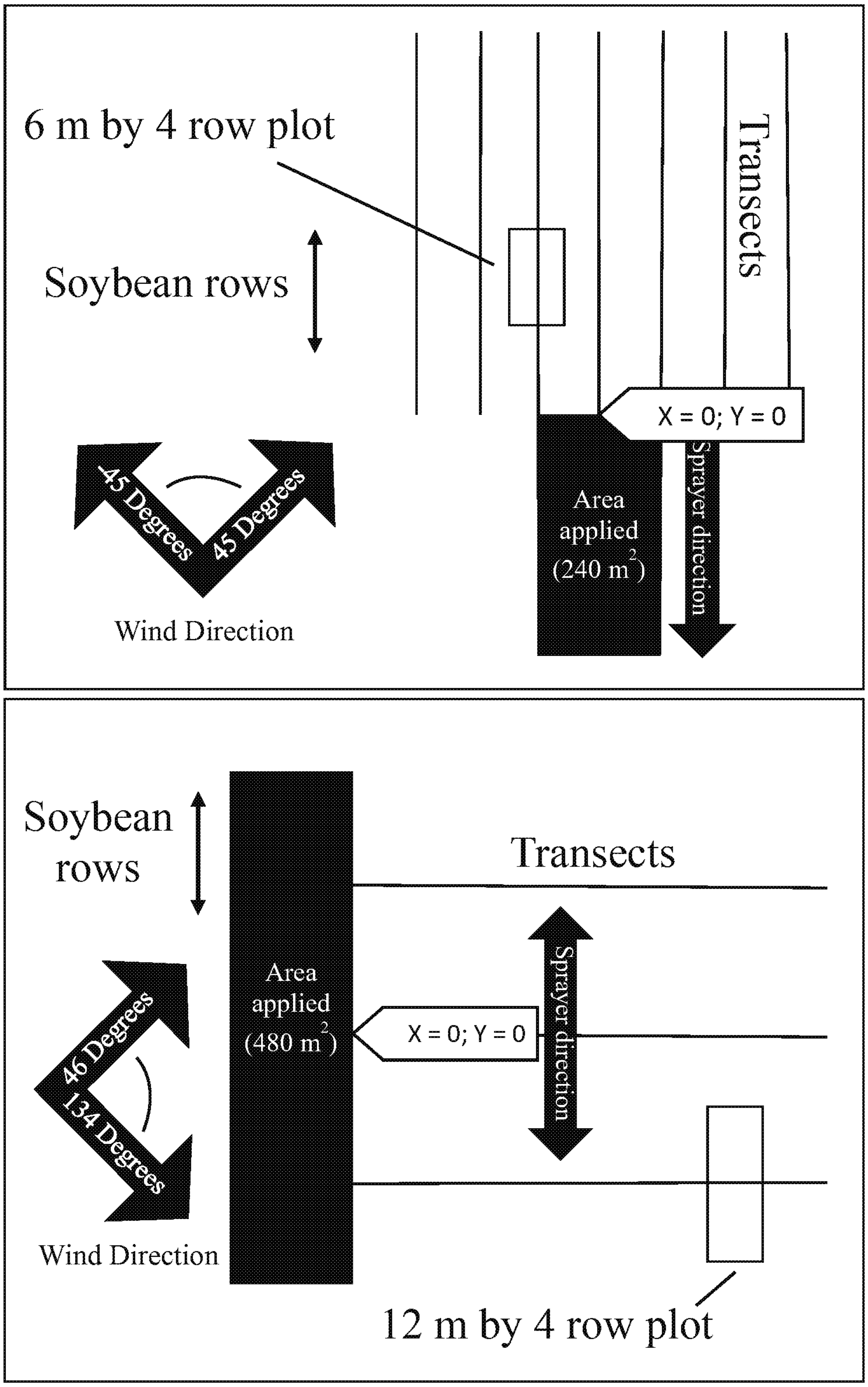

Figure 1. Illustration of drift trial layout for different wind directions.

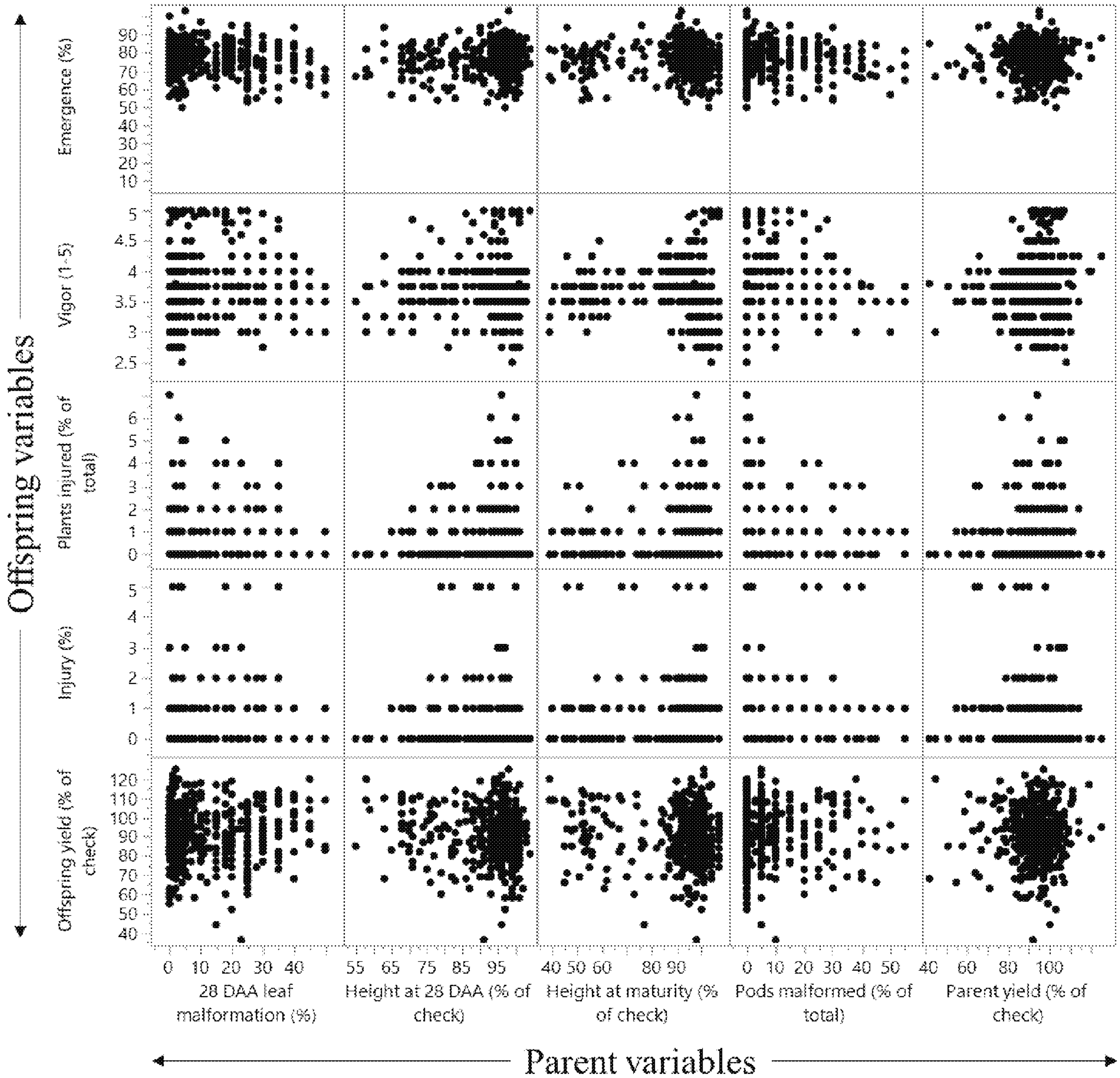

Figure 2. Scatterplot matrix for relationships between parent and offspring variables for R1 drift trials.

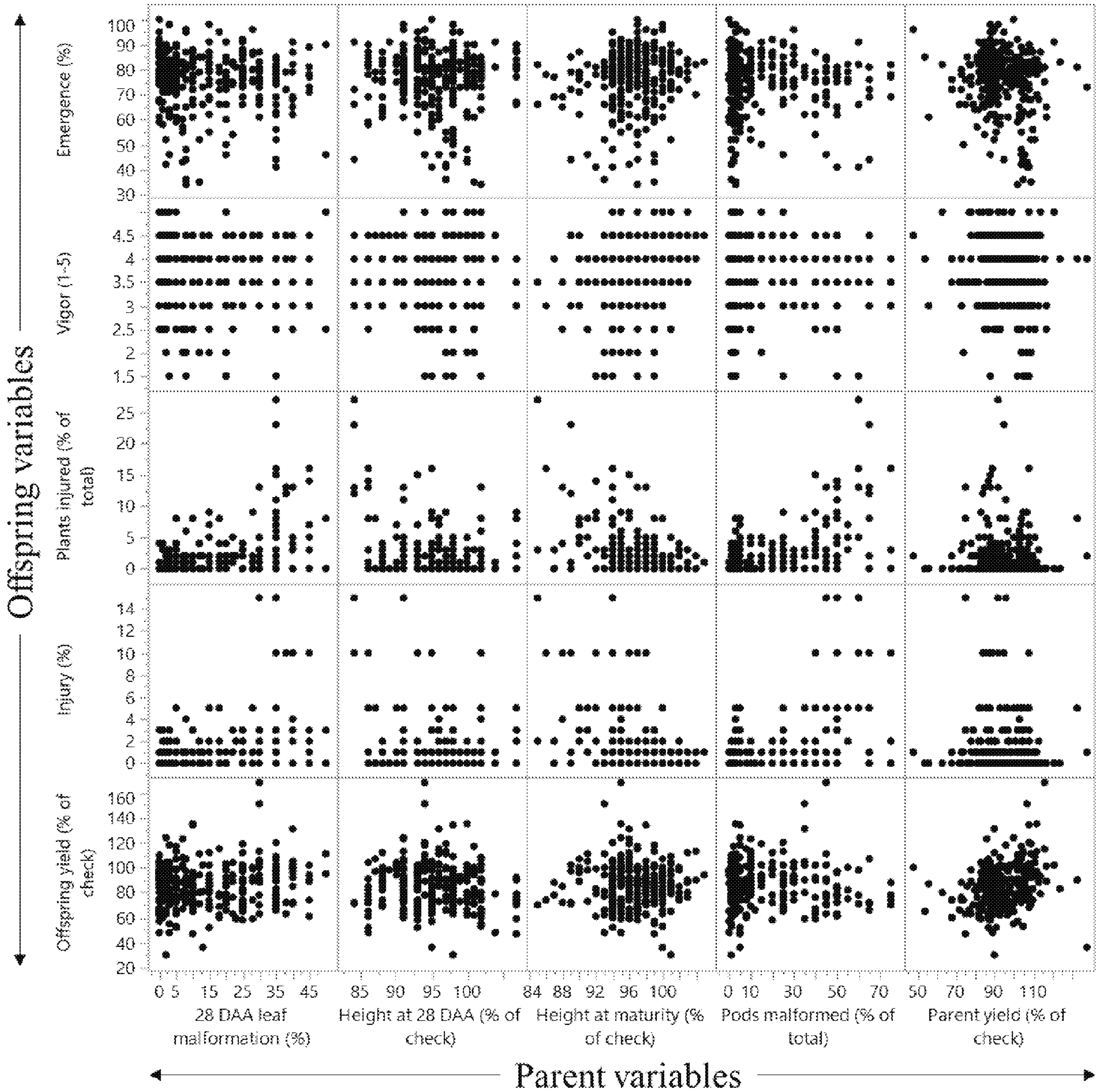

Figure 3. Scatterplot matrix for relationships between parent and offspring variables for R2 drift trials.

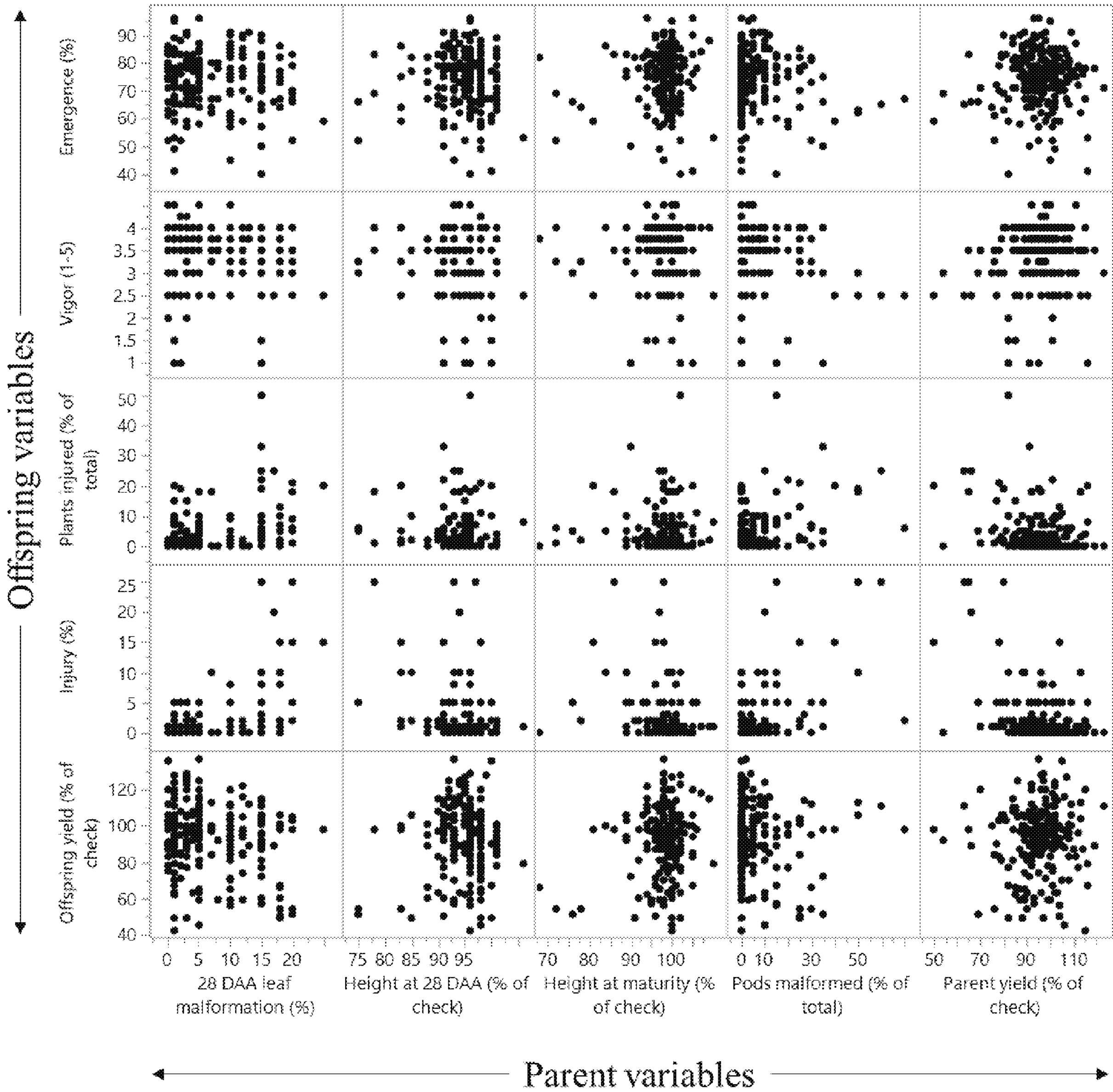

Figure 4. Scatterplot matrix for relationships between parent and offspring variables for R3 drift trials.

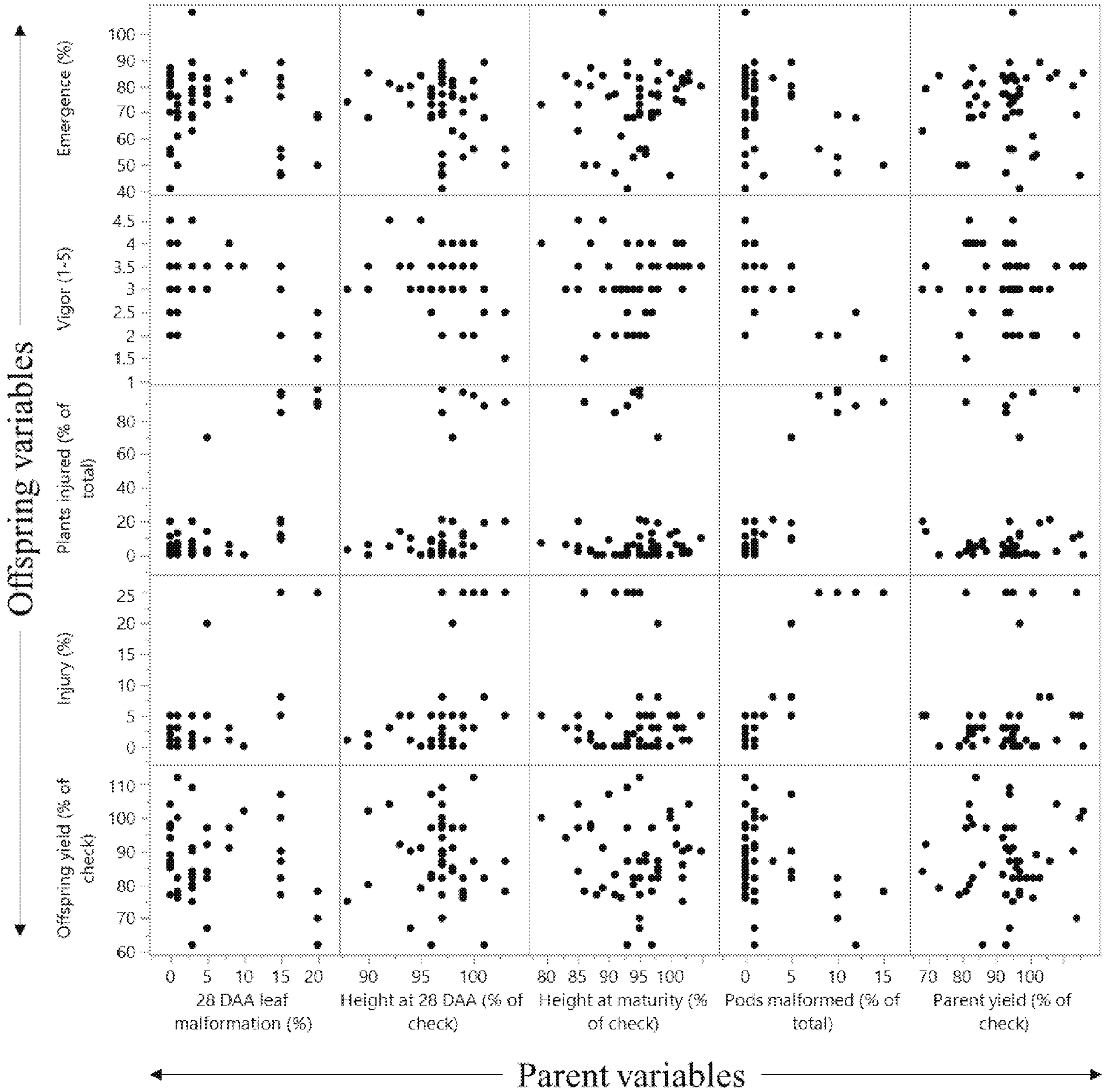

Figure 5. Scatterplot matrix for relationships between parent and offspring variables for R5 drift trials.

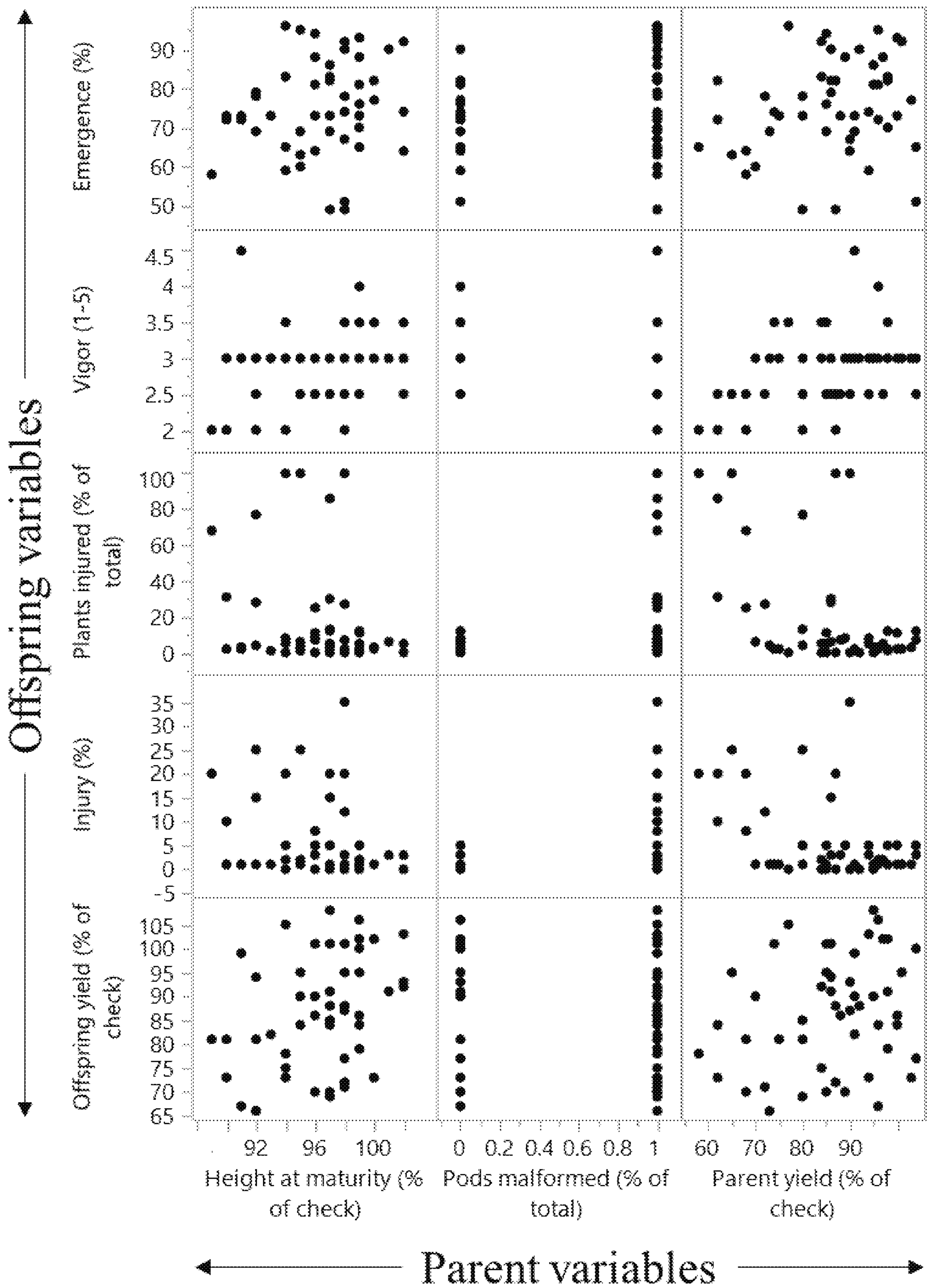

Figure 6. Scatterplot matrix for relationships between parent and offspring variables for R6 drift trials.

Chapter 3

Effect of Low Doses of Dicamba Alone and in Combination with Glyphosate on Parent Soybean and Offspring

Abstract

It is well established that non-dicamba-resistant soybean is highly sensitive to off-target movement of dicamba. However, there is limited knowledge on the effect of low doses of dicamba plus glyphosate mixtures on non-dicamba-resistant soybean – a mixture likely to be used on a vast acreage of dicamba/glyphosate-resistant soybean. Decreased vigor and an expression of dicamba-like symptoms on soybean offspring after exposure to a low dose of dicamba have been established; however, it is unclear if the addition of glyphosate may exaggerate these effects. The objective of this experiment was to examine leaf and pod malformation, along with height and yield effects when dicamba, glyphosate, or a mixture of the two are applied to glufosinate-resistant soybean (non-dicamba-glyphosate-resistant) at sublethal doses. Field applications were made at three growth stages (R1-initial flowering, R3-beginning pod formation, and R5-beginning seed formation) at multiple locations. Two glyphosate rates (1/64 and 1/256 of the labeled rate 870 g ae ha^{-1}) and two dicamba rates (1/64 and 1/256 of the labeled rate 560 g ae ha^{-1}) were used in the study. Adding glyphosate to dicamba increased leaf malformation over dicamba alone when applied at R1. After R3 applications, pod malformation was greater in treatments containing dicamba and glyphosate than dicamba alone. Applications at R5 showed minimal leaf and pod malformation. Seed from field trials were planted in the greenhouse to evaluate the offspring. The number of offspring plants showing dicamba-like symptomology was not increased with the addition of glyphosate to dicamba. Overall, injury to offspring was similar in dicamba alone and dicamba plus glyphosate treatments; however, the number of plants injured increased when parent plants were exposed to sublethal doses of

dicamba at R3 and R5 compared to R1 exposure. Vigor was reduced in dicamba-containing treatments, but not glyphosate-alone treatments. Glyphosate addition to dicamba had no effect on vigor of soybean offspring. Although there is increased injury to parent plants when glyphosate is added to dicamba, this research demonstrates that glyphosate does not contribute to the negative effects of dicamba on soybean offspring.

Nomenclature: Dicamba; glyphosate; soybean, *Glycine max* (L.) Merr.

Key words: Off-target movement, dicamba symptomology, leaf malformation, pod malformation, soybean offspring, dicamba-resistant cotton, dicamba-resistant soybean

Introduction

Dicamba-resistant (DR) cotton (*Gossypium hirsutum* L.) and soybean have been deregulated by the Environmental Protection Agency (EPA) and commercially launched in 2015 and 2016, respectively. Registration of dicamba-containing products (Xtendimax with VaporGrip, Monsanto Corporation, St. Louis, MO; Engenia, BASF Corporation, Research Triangle Park, NC) for over-the-top use in DR soybean and cotton was recently granted for certain states (Anonymous 2016a; Anonymous 2016b). Although a balanced preemergence (PRE) followed by postemergence (POST) herbicide program is recommended, dicamba applied in-crop will add an effective site of action to control problem broadleaf weeds in cotton and soybean (Byker et al. 2013; Flessner et al. 2015; Inman et al. 2016; Spaunhorst and Bradley 2013). However, research involving possible non-target effects of mixtures to be applied in this technology must be studied to examine any negative effects because of reports that dicamba off-target movement has occurred (Barber et al. 2017).

Low-rate exposure or spray tank contamination to non-DR soybean with dicamba can be highly injurious and possibly reduce yield (Auch and Arnold 1978; Boerboom 2004; Solomon and Bradley 2014; Wax et al. 1969; Weidenhamer et al. 1989). With the advent of DR cotton and soybean and approval for use of dicamba in-crop, there will be greater opportunity for damage to susceptible crops. Neighboring fields planted in conventional, glyphosate-resistant, or glufosinate-resistant soybean may be at high risk for injury if dicamba is applied. If sprayers are not properly cleaned following a dicamba application, subsequent spray applications to non-dicamba soybean are likewise expected to damage the crop (Boerboom 2004). Injury symptoms from dicamba exposure to soybean have been previously described mostly as leaf cupping, stem epinasty, and swelling of the stem (Al-Khatib and Peterson 1999; Andersen et al. 2004;

Sciumbato et al. 2004). In addition, pod malformation is a result of low doses of dicamba applied to soybean during reproductive stages (McCown et al. 2016b).

Historically, most dicamba applications occur in late winter or early spring for preplant removal of broadleaf vegetation prior to planting crops or in-crop to V3 to V5 corn, which is at a time when few soybean fields have emerged or emerged plants are in an early vegetative stage. Exposure to dicamba at vegetative stages may result in severe injury, but soybean often recovers from this injury by reproductive stages (Al-Khatib and Peterson 1999; Wax et al. 1969). Soybean compensates for terminal death by initiating branches from the cotyledon and unifoliate axils that reach a height comparable to nontreated plants (Wax et al 1969). These axillary branches produce flowers and pods to offset possible yield reduction from exposure to dicamba (Andersen et al. 2004; Weidenhamer et al. 1989). Therefore, injury resulting from dicamba in vegetative stages may not always result in yield reduction (Al-Khatib and Peterson 1999). Furthermore, the extent of injury may vary due to environmental conditions during and after application (Auch and Arnold 1978; Weidenhamer et al. 1989). Soybean exposed to dicamba when plants are drought stressed will be delayed in recovery when compared to plants experiencing adequate moisture levels (Auch and Arnold 1978; Weidenhamer et al. 1989). For these reasons, the extent of injury to vegetative soybean may not be a good predictor of yield loss because soybean has the ability to recover when exposed to good environmental conditions (Al-Khatib and Peterson 1999; Auch and Arnold 1978).

Applications of dicamba to DR soybean are allowed up to R1 growth stage; therefore, nearby non-DR soybean that are planted at similar dates will also be in reproductive stages (Anonymous 2016a; 2016b). Previous research has examined the effect of dicamba applied at low rates during reproductive development. Yield reduction of 20% required only 4 g ae ha^{-1} when applied at bloom, whereas 35 g ha^{-1} was required for the same yield reduction in vegetative stages (Wax et al. 1969). Furthermore, the dicamba applied at 11 g ha^{-1} at early bloom reduced yield 9 to 42% while not affecting yield at any other growth stage (Auch and Arnold 1978). More recent research also supports the previous claims of Wax et al. (1969) and Auch and Arnold (1978), as they also documented greater yield reduction from dicamba at R2 compared to V3 applications when applied at the same rate (Robinson et al. 2013; Solomon and Bradley 2014). In other research, soybean was 2.5 times more sensitive to yield reduction at R1 growth stage when exposed to dicamba at 4.4 and 17.5 g ha^{-1} than when exposed to the same rates at V3/V4 (Griffin et al. 2013). Previous research may warrant the concern some have over dicamba applications near reproductive non-DR soybean as studies conducted reveal that yield loss is of more concern once soybean reaches flowering stages.

Due to the attempt to achieve broad-spectrum weed control of both grasses and broadleaf weeds in DR crops with a single application, it is likely that glyphosate will be added to the spray tank in most instances. In fact, a premix of dicamba plus glyphosate is being developed for use in DR crops (Roundup Xtend with VaporGrip, Monsanto Corporation, St. Louis, MO). Interactions have been documented concerning the addition of glyphosate to other herbicides in terms of soybean phytotoxicity and weed control. For instance, the addition of glyphosate at 1270 g ha^{-1} to dicamba at 5.6 g ha^{-1} applied at V7 growth stage to glyphosate-resistant/dicamba-sensitive soybean caused 30 to 35% injury compared to 27 to 28% injury when dicamba was applied alone at 2 wk after application (Kelley et al. 2005). Control of glyphosate-resistant tall waterhemp (*Amaranthus rudis* Sauer.) increased when glyphosate was mixed with dicamba (Spaunhorst and Bradley 2013). It was assumed that the effect seen in glyphosate-resistant soybean was because glyphosate slowed the metabolism of dicamba, increasing the intensity and duration of injury over dicamba alone (Kelley et al. 2005); however, no explanation was included in regards to waterhemp control by the tank mixture (Spaunhorst and Bradley 2013).

Dicamba-sensitive soybean exposed to low doses of dicamba at reproductive stages results in offspring that display dicamba-like injury symptoms soon after emergence (Barber et al. 2015; Thompson and Egli 1973). Conversely, for glyphosate, there is no effect on glyphosate-sensitive offspring when low doses of the herbicide are applied during reproductive development to parent plants (Norsworthy 2004). Again, the addition of glyphosate to dicamba increases leaf injury to glyphosate-resistant soybean over dicamba alone (Kelley et al. 2005); however, the effect of low doses of the mixture on offspring needs to be examined.

Previous research has documented glyphosate to be accumulated in bolls of cotton plants when exposed during reproductive growth (Pline et al. 2001); however, research pertaining to growth, maturity, and yield effects of low doses of dicamba plus glyphosate on non-glyphosate/non-DR soybean is limited and needs to be expanded to further to understand potential risks associated with using both herbicides as a mixture or premix in DR crops. Greater soybean yield loss and transmission of dicamba-like symptoms to offspring, have been associated with applications of low doses of dicamba during reproductive development (Auch and Arnold 1978; Barber et al. 2015; Solomon and Bradley 2014; Thompson and Egli 1973; Wax et al 1969). Therefore, an experiment was conducted to examine the effects of low doses of dicamba and glyphosate alone and in combination on non-dicamba/glyphosate-resistant soybean during reproductive development. Subsequently, seed collected from parent plants exposed to dicamba and glyphosate were evaluated to assess the impact of both herbicides alone and in combination on offspring.

Materials and Methods

Field Experiment. Experiments were planted to indeterminate growth habit glufosinate-resistant (glyphosate and dicamba sensitive) soybean on April 30, 2015, and May 4, 2016, at the Arkansas Agriculture Research and Extension Center (AAREC) in Fayetteville, Arkansas, and on May 14, 2016, at the Pine Tree Research Station (PTRS) near Colt, Arkansas. Indeterminate varieties were chosen because previous researchers have documented that the response to dicamba differs between indeterminate and determinate soybean varieties (McCown et al. 2016a). The soil series at PTRS was a Calhoun silt loam (fine-silty, mixed, active, thermic Typic Glossaqualfs) with a pH of 7.8 and 2.23% organic matter. Fields at AAREC were classified as Leaf silt loam (fine, mixed, active, thermic Typic Albaquults) with a pH of 6.1 and 1.75% organic matter. Trials were seeded at 345,800 seeds ha^{-1} with the intention of obtaining a population of 275,000 plants ha^{-1} given 80% germination. At PTRS, soybean was furrow-irrigated and plots at AAREC were irrigated with overhead lateral irrigation. Experiments were irrigated once weekly at 2.5 cm if less than 2.5 cm of rainfall occurred over a 7-d period. Other agronomic information pertaining to each location is provided in Table 1.

Weeds were controlled at the experimental sites with a PRE application of flumioxazin at 70 g ai ha^{-1} at planting followed by two POST applications of glufosinate at 530 g ai ha^{-1} (Liberty, Bayer Cropscience, Research Triangle Park, NC 27709) plus *S*-metolachlor (Dual Magnum, Syngenta Corporation, Greensboro, NC 27408) at 1,064 g ai ha^{-1} added to the first POST application. Treatments were arranged in a randomized complete block (RCB) design with four replications. Dicamba (Clarity, BASF Corporation, Research Triangle Park, NC 27709), glyphosate (Roundup PowerMax, Monsanto Co, St. Louis, MO 63146), or a mixture of the two herbicides was applied at 1/64X (dicamba at 8.75 g ae ha^{-1}, glyphosate at 13.44 g ae ha^{-1}) or 1/256X (dicamba at 2.19 g ha^{-1}, glyphosate at 3.36 g ha^{-1}) of the recommended rate (dicamba at 560 g ha^{-1}, glyphosate at 860 g ha^{-1}) for DR cotton and soybean. Nonionic surfactant was added at 1/64X or 1/256X the full rate of 0.25% v/v (Induce, Helena Chemical Co, Collierville, TN) to all dicamba-alone treatments, but not dicamba plus glyphosate treatments because the glyphosate product already contained an adjuvant. Treatments were mixed using serial dilution from a stock 1X rate, and applications were made on each variety at R1 (initial flower), R3 (initial pod set), and R5 (initial seed formation). All treatments were applied using a handheld boom and CO_2-pressurized backpack sprayer with an output of 143 L ha^{-1} at 270 kPa tipped with 110015 AIXR nozzles (TeeJet Technologies, Springfield, IL 62703). Only the center two rows of each four-row plot were treated. Plot sizes are available in Table 1.

At 2 and 4 wk after application, visual measurements of percent leaf malformation and percent pod malformation were recorded on a scale of 0 to 100%, with 100 being most severe. Canopy height was also recorded at 4 wk after application. At soybean maturity, height (cm) to the terminal of three representative plants was averaged, and final pod malformation ratings were taken. Plots were harvested using a small-plot combine, and soybean grain yield was adjusted to 13% moisture. Canopy height, terminal height, and yield were later converted to a percentage relative to the nontreated control. In addition, a sample of approximately 500 seed from each plot was stored at -10 C after harvest.

Greenhouse Experiment. Seed samples from the previous field experiments were evaluated in a greenhouse at the University of Arkansas Altheimer Laboratory in Fayetteville, Arkansas. Three experiments in total were completed using offspring from both years at AAREC and 2016 from PTRS. Twenty-five seed from each sample were planted at a 2-cm depth into 33- by 18- by 13-cm trays, which were filled with potting mix (Sun Gro Horticulture, Seba Beach, AB, Canada). Trays from each of the four replications were arranged in a RCB design in the greenhouse. The greenhouse was maintained at 32 C daytime and 22 C nighttime temperatures (± 3 C). Natural lighting was supplemented by a metal halide lighting system and set to a 16-h photoperiod. Plants were watered daily to maintain adequate moisture levels. Twenty-one days after planting (DAP), emergence (%), injury (0 to 100% with 0 being no injury and 100 being plant death relative the nontreated control), and number of plants injured were recorded for each tray. Plants were considered injured if they exhibited leaf cupping, leaf strapping, stem epinasty, or stunting, which are common symptoms of soybean exposed to dicamba (Al-Khatib and Peterson 1999; Andersen et al. 2004; Sciumbato et al. 2004). Additionally, plant vigor was rated on a 1 to 5 scale for each tray where was 1 = extremely low vigor (delayed and/or reduced emergence) and 5 = extremely high vigor (seedlings quickly emerged and exhibited normal growth). A standardized rating for vigor has yet to be realized, but the concept of vigor and its importance in crop development are well-accepted (Pollock and Roos 1972). Aboveground biomass was collected at 21 DAP, dried at 66 C for 7 days, and weighed. Percent reduction in biomass was calculated relative to the nontreated control.

Droplet Size Determination. Droplet sizes of all mixtures used in these studies were determined using a Sympatec Helos Vario KR particle size analyzer in a low speed wind tunnel testing at the University of Nebraska West Central Research and Extension Center in North Platte, NE. This system uses laser diffraction to determine droplet size and is accurate from 18 to 3500 microns. Treatments (DGA dicamba alone; glyphosate alone; and the mixture all at 1/64 and 1/256 the proposed use rates of 560 g ae ha^{-1} and 860 g ae ha^{-1}) were repeated three times, and an analysis of variance was performed to evaluate mean Dv50 (point where 50% of the droplets are of the reported size or smaller).

Statistical Analysis. Data from all field and greenhouse trials were subjected to an ANOVA procedure using JMP 12 Pro (SAS Institute, Cary, NC 27511). Site year and replication nested within site year were considered random effects. Soybean growth stage, herbicide treatment, and rate were considered fixed effects. Previous research has documented little to no response by soybean to low rates of glyphosate applied during reproductive development (Norsworthy 2004). In the current experiment, glyphosate treatments caused no response and were excluded from the analysis, thereby reducing the herbicide treatment factor level to two. All remaining data met the assumptions necessary for ANOVA. Main effects and interactions for all dependent variables were assessed. Means were separated using Fisher's protected least significant difference (LSD) test (α=0.05).

Results and Discussion

Soybean Response to Dicamba during Reproductive Development. At 14 d after application (DAA), leaf malformation averaged across rate and timing was greater when glyphosate was added to dicamba (8%) compared to dicamba alone (6%). Applications occurring at R1 growth stage caused more leaf malformation than later timings ($p = 0.012$) (Table 2). In addition, degree of leaf malformation increased with rate; the high rate (dicamba at 8.75 g ha^{-1} alone and with glyphosate at 13.44 g ha^{-1}) produced a 5% increase in leaf malformation compared to the low rate (dicamba at 2.19 g ha^{-1} alone and with glyphosate at 3.36 g ha^{-1}) at this stage when rated 14 DAA. At 28 DAA, an interaction between herbicide and timing was observed ($p = 0.0425$). When applications were made at the R3 and R5 stages, leaf malformation 28 DAA was similar for dicamba alone and dicamba plus glyphosate. However, at 28 DAA of the R1 treatments, addition of glyphosate to dicamba produced a significant 6% increase in leaf malformation compared to dicamba alone. The reason for lack of an effect from glyphosate addition to dicamba at R3 and R5 may be because vegetative growth of soybean has nearly ceased by these stages of development due to floral induction (Heatherly and Elmore 2004). Conversely, during the early stages of reproductive development, soybean is still extending nodes and leaves as floral induction is postponed with indeterminate cultivars (Heatherly and Elmore 2004).

Therefore, dicamba drift to soybean during these stages is more likely to cause leaf malformation than at later reproductive stages.

Visible leaf malformation (injury) resulting from dicamba at 8.75 g ha^{-1} (35%) was somewhat similar to that documented by Kelley et al. (2005) where 38% injury resulted from dicamba at 5.6 g ha^{-1} at 28 DAA during flowering. Solomon and Bradley (2014) observed 15% injury 28 d after treatment with dicamba at 2.8 g ha^{-1}, whereas the current study documented 23% injury at a comparable rate and timing. The extent of injury to soybean from dicamba is known to differ slightly between growth habits, as well as environmental conditions, irrigation practices, and rainfall prior to, during, and after application (Auch and Arnold 1978; McCown et al. 2016a; Wax et al. 1969; Weidenhamer et al. 1989).

In general, extent of leaf malformation decreased as application was delayed. These results are explained by examining soybean plants at each respective stage. During early reproductive stages (R1), vegetative growth is still occurring at a rapid pace under ideal conditions (Heatherly and Elmore 2004). However, once pod formation initiates (R3), vegetative growth slows significantly and nearly ceases once seed formation begins (R5). Therefore, it is not surprising that dicamba exposure to soybean resulted in much greater leaf malformation when plants were still undergoing vegetative growth.

Main effects of both rate ($p = 0.0014$) and timing ($p = 0.0001$) were observed at 14 DAA (Table 3). Pod malformation was 6% higher with the low rate than with the high rate, averaged over herbicide and timing. Applications at R3 resulted in the greatest pod malformation (11%). At 28 DAA of the R3 treatments, pod malformation increased with the addition of glyphosate to dicamba. Furthermore, pod malformation was also dependent on both rate and application timing. The greatest pod malformation (29%) was documented among treatments involving high rates at R3 growth stage. Little pod malformation was observed after R5 applications (2 to 5%).

At soybean maturity, pod malformation involved interactions of herbicide by timing and rate by timing ($p= 0.0033$; $p= <0.0001$). Pod malformation at soybean maturity resulting from application at R1 and R5 was similar. However, the addition of glyphosate to dicamba increased pod malformation by 10% when applied at R3 growth stage (Table 3). When averaged across herbicide, pod malformation was greatest after application of the high rate at R3 growth stage (47%). This timing by rate combination was significantly greater than the low rate at this timing (23%) as well as all other combinations.

Extent of pod malformation has not been quantified in previous research. However, pod malformation occurs following dicamba drift (Auch and Arnold 1978; Weidenhamer et al. 1989). In the present study, the greatest percentage of pod malformation followed applications to R3 soybean. The focus of soybean at the R3 growth stage is to initiate pod formation; therefore, exposure to dicamba will have the greatest possibility of generating severe pod malformation. Dicamba exposure to soybean at R1 caused severe leaf malformation; however, pod formation has not yet begun at this timing. Hence, soybean plants have time to recover from dicamba exposure, which may lead to a lower dicamba concentration in the plant before pod formation begins and consequently result in a lower percentage of malformed pods. By the time seed formation stages (R5-R6) are reached, pod formation has been completed in all but the top nodes of soybean plants. In the current study, pod malformation after a low dose of dicamba at R5 was minimal (0 to 5%) and only documented in the upper two to four nodes.

When averaged across rates, glyphosate alone did not reduce 28 DAA canopy or mature terminal height of soybean at any timing relative to the nontreated check at 28 DAA or maturity (Table 3). Canopy height at 28 DAA was reduced most by dicamba (24%) and dicamba plus glyphosate (26%) when applied at R1 growth stage (Table 3). Application of dicamba and dicamba plus glyphosate to soybean at R3 resulted in canopy height reductions of 14% and 10%, respectively. Application of herbicides at R5 did not reduce soybean canopy height compared to the nontreated check.

At soybean maturity, height to the terminal node displayed a main effect of rate and an interaction between herbicide and timing ($p= 0.0261$; $p= <0.0001$) (Table 3). The high rate of dicamba plus glyphosate (1/64X) reduced terminal height 14%, whereas the low rate of the combination (1/256X) caused a significantly lower reduction of 10%. When averaged across rates, dicamba and dicamba plus glyphosate applied at R1 reduced plant heights more than any other herbicide by timing combination. Dicamba and dicamba plus glyphosate applied at R3 were similar, with terminal height reductions of 12 and 14%, respectively. Canopy heights of plants treated at R5 were minimally affected by any treatment combination. In general, height reductions decreased as dicamba applications were delayed. This study suggests that dicamba exposure to soybean in early flowering stages results in the greatest height reduction among applications during reproductive development, as has been reported in other research (Auch and Arnold 1978; Solomon and Bradley 2014; Weidenhamer et al. 1989). The lack of height reductions at later stages is likely because soybean plants shift to pod and seed production and plants are already near maximum height.

Delay in maturity was minimal in the present study, with no treatment resulting in more than a 4-d delay in maturity (Table 3). The present study uses rates similar to ones used in previous studies, which showed comparable delays in soybean maturity occurring at these rates (Solomon and Bradley 2014; Wax et al. 1969). In other research, delays in soybean maturity increased with dicamba rate (Auch and Arnold 1978; Kelley et al. 2005; Wax et al. 1969). Auch and Arnold (1978) reported delays in soybean maturity to range from 3 to 19 days when dicamba at 11 to 56 g ha^{-1} was applied at reproductive stages. Comparable delays (4 to 24 d) were reported when 2 to 64 g ha^{-1} were applied in bloom stages (Wax et al. 1969).

Soybean grain yield reduction involved both herbicide by timing and rate by timing interactions ($p= <0.0001$; $p= 0.0087$) (Table 3). Glyphosate applications did not reduce yield at any timing compared to the nontreated control, which agrees with previous research by Norsworthy (2004) where glyphosate at 8 g ha^{-1} applied at R2 or R5 stages did not reduce yield. The greatest yield reductions were from dicamba alone or with glyphosate applied at R1 growth stage, which has been reported previously (Wax et al. 1969; Solomon and Bradley 2014; Auch and Arnold 1978). Yield reductions from R3 applications of dicamba (7%) and dicamba plus glyphosate (6%) were small but were greater than the nontreated check. Applications during seed fill (R5) did not reduce yield compared to the nontreated check. Yield reduction was present only in treatments where height reduction at maturity occurred. Soybean yield reduction following mature height reduction has been documented previously (Weidenhamer et al. 1989).

Effect of Soybean Exposure to Dicamba on Offspring. Emergence of soybean offspring was significant for the main effects of herbicide ($p= 0.003$) and rate ($p=0.0481$) (Table 4). Glyphosate added to dicamba had no effect on offspring emergence relative to dicamba alone; however, dicamba-containing treatments lowered emergence by as much as 3% compared to the nontreated check. Soybean emergence from plants treated with the lowest rate was 100%. High rates decreased emergence 2%, which is likely not of biological importance and would not be noticed at a commercial production scale. Ideal growing conditions in the greenhouse may have expedited seed emergence over less-than-ideal field environments. Previous research using higher rates of dicamba applied during reproductive development showed reductions in germination and emergence (Thompson and Egli 1973; Wax et al. 1969). Germination was not affected by rates similar to those used in this study; yet, Wax et al. (1969) reported that germination was reduced to 79 and 19% when 1/32 (17.5 g ha^{-1}) and 1/16 X (35 g ha^{-1}) rates were applied. Emergence was only 50% when dicamba at 30 g ha^{-1} was applied, and soybean offspring failed to emerge when dicamba at 220 g ha^{-1} was applied during flowering stages (Thompson and Egli 1973).

Soybean plants exposed to a low dose of dicamba at R5 growth stage were more likely to experience a high percentage of injured offspring; however, adding glyphosate to dicamba did not increase injury to the offspring (Table 5). A rate by timing interaction was observed, with the highest percentage of injured plants (96%) resulting from parent plants treated with the high rates of dicamba alone and including glyphosate applied at R5 growth stage (p=0.0026). The low rates applied at R5 reduced incidence of emerged soybean offspring injury (dicamba-like symptoms), but only to 81%. Applications of high and low rates at R3 resulted in 59 and 34% of offspring being malformed, respectively. No difference was observed in percentage of plants malformed between high and low rates applied at R1, and symptoms were less than other combinations of rate and timing.

Overall, percentage of plants malformed and the degree of leaf malformation increased as application to soybean was delayed (Table 5), likely because application at late reproductive stages allowed for more dicamba storage in the seed. Dicamba exposure during reproductive development may allow offspring emergence, but with many of the emerged plants having malformed leaves. If auxin-like symptomology arises in newly planted soybean fields, growers may have cause for concern. In severe cases, the auxin-like symptomology could be mistaken as drift or carryover of auxin herbicides, causing growers to blame neighbors or custom applicators.

Reductions in vigor generally increased with later applications for all treatments, except for glyphosate alone, which maintained vigor at all applications and rates (Table 6). The addition of glyphosate to dicamba did not significantly reduce soybean offspring vigor at any growth stage compared to dicamba alone. Vigor reduction to offspring from dicamba-containing solutions applied at R1 ranged from 11 to 12%, regardless of rate. Treatment with dicamba-containing solutions at R3 resulted in reduced vigor to offspring ranging from 15 to 20% but did not differ between rates.

Application of dicamba at seed fill (R5) had the greatest impact on offspring vigor (Table 6). Dicamba and dicamba plus glyphosate applications at the low rate caused 22 and 30% reductions in vigor. Vigor was reduced more from the high rate of dicamba-containing solutions applied at R5 than from any other treatment.

Reduction in soybean offspring biomass for glyphosate-alone treatments was minimal (0 to 6%) (Table 6). The addition of glyphosate to dicamba did not further decrease biomass. Dicamba and dicamba plus glyphosate treatments caused similar biomass reduction when applied at R1 and R3, with values ranging from 4 to 8%. Trends for this parameter generally followed vigor reductions, as the greatest offspring biomass reduction occurred from the R5 application. At this timing, the lowest rate of dicamba alone and dicamba plus glyphosate resulted in 9 and 14% reduction in offspring biomass. At the higher rate, application of dicamba alone led to a 34% reduction, and the addition of glyphosate reduced biomass to 36% of the untreated check.

These results document that dicamba exposure to soybean at R5 growth stage can decrease vigor of offspring by as much as half and biomass up to a third. Knowing that the rates used in these experiments will not always cause noticeable injury at R5 growth stage is worrisome concerning soybean seed production fields as drift or tank contamination during seed fill could go unnoticed. Furthermore, standard germination tests may not identify poor quality seed as dicamba-containing solutions only slightly reduced emergence (2 to 3%) in this study. Therefore, contaminated seed may not be identified and subsequently be distributed to growers.

Practical Implications. The addition of an alternative site of action will increase diversity in soybean and cotton weed control programs. However, the addition of a grass-controlling herbicide such as glyphosate must be included in dicamba-glyphosate-resistant cropping systems for broad-spectrum weed control. Yet, precautions must be taken to reduce the chance of off-target movement to susceptible crops. Research herein and previous research show that extremely low doses of dicamba are harmful to soybean growth, and effects may be transmitted to offspring (Wax et al. 1969; Thompson and Egli 1973; Auch and Arnold 1978). Increased leaf or pod malformation caused by glyphosate addition to dicamba will not further reduce yields over a comparable dose of dicamba alone. However, predicting soybean yield loss by visual injury may not be ideal in reproductive stages as models often overestimate yield loss (Egan et al. 2014).

The addition of glyphosate to dicamba may lead to increased leaf and pod malformation to soybean after drift occurs; yet, observations on offspring such as emergence, malformation, and biomass are similar to those of dicamba alone. To investigate possible differences in herbicide mixtures that may be causing the effect seen on soybean exposed to drift, droplet size analysis was performed at the University of Nebraska West Central Research and Extension Center in North Platte, NE. Droplet sizes of the herbicide treatments evaluated were not different (data not shown); therefore, droplet size played no role in the effects seen in parent plants. Further research must be completed to determine if glyphosate is aiding in the translocation of dicamba to cause the observed effect in parent plants. The addition of glyphosate to dicamba did numerically increase vigor and biomass reductions to offspring. However, these differences were not statistically significant in this study. This research does conclude that seed fill exposure of soybean to dicamba will lead to greater offspring reductions in vigor and biomass; therefore, further research completed during seed fill using additional rates of glyphosate and dicamba may detect significant differences.

Injury observed to parents from soybean exposure to low doses of dicamba at seed fill was minimal. Therefore, it may be possible that dicamba exposure to soybean could go unnoticed. An additional concern would be that dicamba exposure to seed production fields might go unnoticed and continue through the harvest, cleaning, and bagging processes. Identification of seed contaminated by dicamba may be difficult. Testing of seed for presence of dicamba through laboratory analysis could prove costly. These experiments document that germination and emergence may not be reduced when dicamba at 2.19 and 8.75 g ha^{-1} are applied in reproductive development. However, effects are seen in these plants after emergence. A reduction in biomass could in turn lead to the necessity for additional weed control measures as plants may be delayed in canopy formation. Additional training may be helpful for commercial applicators involved in DR cropping systems as not all are aware of the care that needs to be taken when applying dicamba (Bish and Bradley 2017). Dicamba application training is crucial in informing the uninformed to guard against economic loss incurred by growers not planting DR soybean, contingent upon physical drift being solely responsible for off-target damage.

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Table 1. Cultivars, plot sizes, planting dates and application dates for experiments conducted in Fayetteville and Pine Tree, Arkansas.

			Herbicide			Application dates		
Location	Year	Cultivar	technology	Plot size (m)	Planting date	R1	R3	R5
Fayetteville	2015	Pioneer 95L01	LibertyLink	3.7 × 6.1	4/30/2015	7/6/2015	7/26/2015	8/12/2015
Fayetteville	2016	Pioneer 49T31	LibertyLink	3.7 × 7.6	5/4/2016	7/9/2016	7/22/2016	8/10/2016
Pine Tree	2016	Progeny 4814	LibertyLink	3.1 × 6.1	6/9/2015	7/20/2016	7/30/2015	8/24/2016

Table 2. Anova table for field experiments.

Variable	Source	DF	F Ratio	Prob > F
Leaf malformation at 14 DAA	Herbicide	1	4.8843	0.0292
	Rate	1	1.2465	0.2667
	Stage	2	93.8031	<.0001
	Herbicide*Rate	1	0.0239	0.8774
	Herbicide*Stage	2	2.9146	0.0586
	Rate*Stage	2	4.6141	0.012
	Herbicide*Rate*Stage	2	0.3747	0.6884
Pod malformation at 14 DAA	Herbicide	1	1.2483	0.2674
	Rate	1	11.041	0.0014
	Stage	2	10.3411	0.0001
	Herbicide*Rate	1	0.1771	0.6751
	Herbicide*Stage	2	0.0735	0.9292
	Rate*Stage	2	2.8476	0.0641
	Herbicide*Rate*Stage	2	0.0154	0.9848
Leaf malformation at 28 DAA	Herbicide	1	5.0993	0.0263
	Rate	1	6.3456	0.0135
	Stage	2	214.2049	<.0001
	Herbicide*Rate	1	0.0061	0.9381
	Herbicide*Stage	2	3.2709	0.0425
	Rate*Stage	2	12.6905	<.0001
	Herbicide*Rate*Stage	2	0.509	0.6028
Height at 28 DAA	Herbicide	2	25.5571	<.0001
	Rate	1	2.1996	0.1403
	Stage	2	38.297	<.0001
	Herbicide*Rate	2	0.5611	0.5719
	Herbicide*Stage	4	10.5466	<.0001
	Rate*Stage	2	0.5256	0.5924
	Herbicide*Rate*Stage	4	0.4598	0.7651
Height at maturity	Herbicide	2	36.2868	<.0001
	Rate	1	5.0302	0.0261
	Stage	2	65.5059	<.0001
	Herbicide*Rate	2	1.5975	0.2051
	Herbicide*Stage	4	17.0167	<.0001
	Rate*Stage	2	1.8015	0.1679
	Herbicide*Rate*Stage	4	0.3009	0.8771

Table 2 continued

Variable	Source	DF	F Ratio	Prob > F
Mature pod malformation	Herbicide	1	6.2233	0.014
	Rate	1	69.2643	<.0001
	Stage	2	198.0152	<.0001
	Herbicide*Rate	1	0.0281	0.8671
	Herbicide*Stage	2	5.9765	0.0033
	Rate*Stage	2	24.3828	<.0001
	Herbicide*Rate*Stage	2	0.8432	0.4328
Maturity delay	Herbicide	2	11.1277	<.0001
	Rate	1	0.1487	0.7005
	Stage	2	17.0815	<.0001
	Herbicide*Rate	2	0.1487	0.862
	Herbicide*Stage	4	5.62	0.0004
	Rate*Stage	2	1.5142	0.2242
	Herbicide*Rate*Stage	4	0.9953	0.413
Yield	Herbicide	2	8.3847	0.0003
	Rate	1	11.4513	0.0009
	Stage	2	22.8636	<.0001
	Herbicide*Rate	2	2.563	0.0798
	Herbicide*Stage	4	8.5739	<.0001
	Rate*Stage	2	4.8683	0.0087
	Herbicide*Rate*Stage	4	1.0891	0.3633

Abbreviations: DAA = days after application; DF = degrees of freedom

Table 3. Leaf malformation, pod malformation, height, maturity delay, and yield of soybean when exposed to dicamba and glyphosate applied at two rates during R1, R3, and R5 growth stages.[ab]

	Leaf malformation[c]		Pod malformation[c]		Relative height			
Treatment	14 DAA	28 DAA	28 DAA	Maturity	28 DAA	Maturity	Maturity delay	Relative yield
Herbicide × Timing	----------	----------	----------	%	----------	----------	d	%
glyphosate × R1	-	-	-	-	100 a	96 a	2 b	100 a
dicamba × R1	-	29 b	-	12 c	76 c	68 c	2 b	82 c
dicamba + glyphosate × R1	-	35 a	-	13 c	74 c	67 c	2 b	84 c
glyphosate × R3	-	-	-	-	100 a	98 a	1 b	98 ab
dicamba × R3	-	9 c	-	30 b	86 b	88 b	2 b	93 b
dicamba + glyphosate × R3	-	10 c	-	40 a	90 b	86 b	4 a	94 b
glyphosate × R5	-	-	-	-	101 a	95 a	1 b	98 ab
dicamba × R5	-	1 d	-	4 d	101 a	98 a	4 a	101 a
dicamba + glyphosate × R5	-	1 d	-	3 d	102 a	96 a	2 b	101 a
Rate × Timing								
1/256 X × R1	14 b	27 b	8 cd	10 d	-	-	-	94 b
1/64 X × R1	19 a	37 a	15 b	15 c	-	-	-	84 c
1/256 X × R3	8 c	10 c	12 bc	23 b	-	-	-	97 ab
1/64 X × R3	6 c	8 c	29 a	47 a	-	-	-	93 b
1/256 X × R5	< 1 d	1 d	2 e	2 e	-	-	-	100 a
1/64 X × R5	< 1 d	1 d	5 de	5 de	-	-	-	100 a

[a] Means followed by the same letter within a column are not significantly different using Fisher's protected LSD ($\alpha = 0.05$).

[b]A 1X rate of dicamba and glyphosate was 560 and 870 g ae ha^{-1}, respectively.

[c] Leaf and pod malformation averages for glyphosate-containing treatments were not included due to lack of soybean response.

Table 4. ANOVA table for greenhouse experiments.

Variable	Source	DF	F Ratio	Prob > F
Emergence (% of nontreated)	Herbicide	2	6.0073	0.003
	Rate	1	3.9618	0.0481
	Herbicide*Rate	2	0.5134	0.5993
	Timing	2	0.1064	0.8991
	Herbicide*Timing	4	0.7443	0.563
	Rate*Timing	2	1.7885	0.1702
	Herbicide*Rate*Timing	4	0.9508	0.436
Plants injured (%)	Herbicide	2	2.1202	0.1481
	Rate	1	21.1886	<.0001
	Herbicide*Rate	2	0.0788	0.7794
	Timing	2	213.8425	<.0001
	Herbicide*Timing	4	1.6643	0.1938
	Rate*Timing	2	6.2854	0.0026
	Herbicide*Rate*Timing	4	0.0795	0.9236
Injury (% of nontreated)	Herbicide	2	1.4364	0.2332
	Rate	1	31.9317	<.0001
	Herbicide*Rate	2	0.0549	0.8151
	Timing	2	134.2254	<.0001
	Herbicide*Timing	4	0.5748	0.5644
	Rate*Timing	2	20.9325	<.0001
	Herbicide*Rate*Timing	4	0.7616	0.4692
Vigor (1 to 5)	Herbicide	2	54.644	<.0001
	Rate	1	11.0772	0.0011
	Herbicide*Rate	2	1.9344	0.1475
	Timing	2	43.6453	<.0001
	Herbicide*Timing	4	10.9328	<.0001
	Rate*Timing	2	6.0258	0.0029
	Herbicide*Rate*Timing	4	3.986	0.004
Biomass (% of nontreated)	Herbicide	2	18.4657	<.0001
	Rate	1	17.8643	<.0001
	Herbicide*Rate	2	1.7145	0.183
	Timing	2	22.44	<.0001
	Herbicide*Timing	4	8.9446	<.0001
	Rate*Timing	2	6.8346	0.0014
	Herbicide*Rate*Timing	4	5.4281	0.0004

Abbreviations: DF = degrees of freedom

Table 5. Percentage of plants injured and intensity of leaf malformation documented in offspring whose parents were exposed to low rates of glyphosate and dicamba during reproductive development.[ab]

Rate × Timing	Plants injured [c]		Visible leaf malformation [c]	
	------------------------%------------------------			
1/256 X × R1	15	e	4	d
1/64 X × R1	15	e	2	d
1/256 X × R3	34	d	4	d
1/64 X × R3	59	c	8	c
1/256 X × R5	81	b	13	b
1/64 X × R5	96	a	26	a

[a] Means followed by the same letter within a column are not statistically different using Fisher's protected LSD ($\alpha = 0.05$).

[b]A 1X rate of dicamba and glyphosate was 560 and 870 g ae ha^{-1}, respectively.

[c] Percentage of plants injured and visible leaf malformation ratings for glyphosate-only treatments were not included because no response was observed.

Table 6. Relative vigor and biomass reduction documented in offspring whose parents were exposed to low rates of glyphosate and dicamba during reproductive development. [a]

	Relative vigor reduction						Relative biomass reduction					
	1/256 of use rate[b]			1/64 of use rate[b]			1/256 of use rate[b]			1/64 of use rate[b]		
Herbicide	R1	R3	R5	R1	R3	R5	R1	R3	R5	R1	R3	R5
	% of non-treated											
Glyphosate	3a	3ab	7a-d	4abc	8a-e	3ab	0a	1ab	2ab	4ab	6abc	0a
Dicamba	11a-f	19fgh	22hi	12b-g	20gh	44j	9bc	2ab	9bc	5abc	8bc	34d
Dicamba + glyphosate	12c-g	15d-h	30i	11a-f	16e-h	50j	4ab	5ab	14c	6abc	5ab	36d

[a] Means followed by the same letter within relative vigor reduction and relative biomass reduction are not statistically different using Fisher's protected LSD ($\alpha = 0.05$).

[b] Fraction of full labeled rate (560 g ae ha^{-1} of dicamba and 870 g ae ha^{-1} of glyphosate).

139

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Chapter 4

Comparison of Off-target Movement from DGA and BAPMA Dicamba to Non-dicamba-resistant Soybean

Abstract

It is well established that dicamba can cause severe injury to non-dicamba-resistant soybean. The availability of dicamba-resistant soybean and cotton varieties, in conjunction with release of new dicamba formulations approved for over-the-top use in these crops occurred in 2016. Until this approval, use of dicamba was limited to a relatively small amount of corn acres in the summer months when temperatures are conducive for volatility. Hence, studies were conducted in 2015 and 2016 at the Northeast Research and Extension Center in Keiser, AR, to examine the primary and secondary movement of two dicamba formulations using non-dicamba-resistant soybean as a bio-indicator. Diglycolamine (DGA) and N,N-Bis-(3-aminopropyl) methylamine (BAPMA) dicamba were applied simultaneously at 560 g ae ha^{-1} in the center of two side-by-side 8-ha fields to vegetative glufosinate-resistant soybean. On the same day, a rate response experiment was established encompassing nine different dicamba rates of each formulation. Results from the rate response experiment indicate that soybean is equally sensitive to DGA and BAPMA dicamba. Six to eight hours after application of the large drift trial in 2015, a rain event occurred likely limiting volatility by incorporating some of the herbicide into the soil. As a result, secondary drift was less in 2015 than in 2016. However, minimal secondary injury (< 5%) occurred 12 m further into DGA dicamba plots in 2015. In 2016, secondary movement was decreased by 72 m when BAPMA dicamba was used compared to DGA dicamba.

Nomenclature: dicamba; soybean, *Glycine max* (L.) Merr.

Key words: Off-target movement, primary drift, secondary drift, volatility

Introduction

Cotton and soybean cultivars with resistance to the synthetic-auxin dicamba have been commercially launched and are now widely available for purchasing and planting by growers. This new biotech trait will allow dicamba to be sprayed postemergence (POST) over these crops, which will range from April through August in some areas of the country (USDA-NASS 2010). Dicamba provides excellent control of some key broadleaf weed species, including glyphosate-resistant horseweed [*Conyza canadensis* (L.) Cronq.] (Kruger et al. 2010) and giant ragweed (*Ambrosia trifida* L.) (Vink et al. 2012). Although in-crop applications of dicamba in dicamba-resistant (DR) soybean and cotton will not be as broad-spectrum as glyphosate once was, it will provide a new site of action to be used in these crops to improve weed control and guard against herbicide resistance if used responsibly. Yet, integrating a new site of action into a herbicide program may only delay herbicide resistance and integrating non-herbicidal options may provide the best insurance against herbicide resistance (Harker et al. 2017).

Dicamba is a member of the benzoic acid family of herbicides but more widely grouped as a synthetic auxin because it mimics indole acetic acid (Mithila et al. 2011). For over 50 years, dicamba has been used for broadleaf weed control in corn (*Zea mays* L.), small grains, and pastures. Despite over five decades of use, only two weeds, kochia (*Kochia scoparia* L. Schrad.) and prickly lettuce (*Latuca serriola* L. Lacse), have evolved resistance to dicamba in the United States (Heap 2017). As with other pesticides, dicamba may move off-target by primary (physical) drift at the time of application. Dicamba is also a volatile compound, and secondary (volatile) movement and injury to soybean via volatilization can occur (Behrens and Lueschen 1979; Egan and Mortensen 2012; Mueller et al. 2013). Early research documented the volatile component of dimethylamine (DMA) dicamba to be free dicamba acid (Behrens and Lueschen 1979).

Incorporation of dicamba into a POST dicamba-resistant (DR) soybean or cotton weed control program will enable its use to be expanded into summer months where temperatures may reach yearly maximums. As with other herbicides, volatility of dicamba increases with temperature (Grover 1975; Behrens and Lueschen 1979), which is a concern for growers making applications under warm conditions. Furthermore, when high temperature is paired with low humidity, volatile losses may increase as there is more opportunity for dicamba acid to convert to a gaseous state.

Early research reported that after application of the DMA salt of dicamba, volatilization can occur at least 3 days after application (Behrens and Lueschen 1979). However, dicamba at only 280 g ae ha^{-1} (half the current rate of 560 g ae ha^{-1} for DR crops) was used. Soybean injury was greatest for plants placed in the field the day of the application, and decreased the following 2 days as different sets of plants were exposed. Symptoms decreased as potted plants were placed further from the application area; yet, injury still occurred to soybean placed 60 m from the application. Furthermore, it is possible that soybean injury from volatile loss of dicamba could be increased both in intensity and distance from the application if dicamba is applied to a larger area as only a 30- by 30-m area was sprayed in this research.

Previous researchers have shown DGA dicamba to be less volatile than DMA dicamba under field conditions (Egan and Mortensen 2012; Mueller et al. 2013); albeit, recent research found DGA dicamba volatilizes for at least 3 days after application (Anonymous 2017). Air samplers documented a 50% decrease in detection of gaseous dicamba over plots that received DGA dicamba as opposed to the DMA formulation (Mueller et al. 2013). When using bioassay soybean plants to estimate the amount of dicamba leaving the application area via secondary drift, off-target movement was reduced by 94% when the DGA salt of dicamba was applied over the DMA salt (Egan and Mortensen 2012). Although injury to soybean from secondary drift of DGA dicamba was less than that of DMA dicamba, malformation was still noticed out to 20 m in multiple trials when treating only 335 m^2 (0.033 ha). Therefore, use of this formulation in DR crops may need to be accompanied by buffers on all sides of the application area to guard against off-target movement because secondary movement could cause damage to multiple sides of a field if winds shift direction within 3 days of application.

The most recently labeled dicamba formulations for use in DR soybean and cotton are thought to have reduced volatile losses; however, little published research has been compiled on these new formulated products. As of November 9, 2016, a DGA dicamba with an additive (XtendiMax with VaporGrip, Monsanto Company, St. Louis, MO) was approved for supplemental labeling for use in DR cotton and soybean in the United States (Anonymous 2016a). This formulation is a combination of the previously available diglycolamine (DGA) salt of dicamba and acetic acid as an additive that is said to reduce volatile loss by inhibiting formation of free dicamba acid (MacInnes 2017). Additionally, the N,N-Bis-(aminopropyl) methylamine (BAPMA) salt of dicamba (Engenia, BASF Corporation, Research Triangle Park, NC 27709) was granted supplemental registration at a later date (Anonymous 2016b). This salt of dicamba is also purported to have reduced volatility over previous forms (Westberg and Adams 2017).

Although BAPMA dicamba is purported to have decreased secondary loss via volatilization over previous forms, published field research documenting the lower risk of this formulation does not exist. Previous research aimed at comparing volatile losses from herbicides either used potted bioassay plants that were not experiencing field soil conditions, or sought to quantify by analytical methods only the amount of herbicide leaving the application area (Bauerle et al. 2015; Egan and Mortensen 2012; Sciumbato et al. 2004; Strachan et al. 2013). Furthermore, if the size of the application area directly correlates to the amount of volatile loss, commercial applications to larger fields may result in a greater amount of secondary injury to soybean than previously realized. Therefore, a field experiment was designed to examine possible differences between DGA and BAPMA dicamba after application using commercial application techniques.

Materials and Methods

Drift Experiments. Field experiments were conducted in 2015 and 2016 at the Northeast Research and Extension Center in Keiser, AR. Glufosinate-resistant soybean (Bayer Credenz 4950LL) was planted in two adjacent 8-ha fields on June 15, 2015, and June 13, 2016. Rows were bedded on 97-cm centers. Weed control was provided with preemergence (PRE) applications of flumioxazin at 71 g ai ha^{-1} plus paraquat at 701 g ai ha^{-1} and two POST applications of glufosinate at 595 g ai ha^{-1} plus clethodim at 76 g ai ha^{-1}. Furrow irrigation was used to supplement natural rainfall.

A 38- by 38-m area (0.144 ha) in the center of each field simultaneously received either DGA or BAPMA dicamba applied at 560 g ae ha^{-1} with one of two Bowman Mudmaster (Bowman Manufacturing, Newport, AR, 72112) high-clearance sprayers. Applications were made at soybean V6/V7 in 2015 and V4/V5 growth stage in 2016. Each sprayer was equipped with a broadcast boom having a 7.6-m swath tipped with 11003 TTI nozzles (TeeJet Technologies, Springfield, IL) calibrated to deliver 94 L ha^{-1} at 275 kPa while traveling at 15 km h^{-1}. Five passes were made, with each sprayer (one for each formulation) simultaneously applying the herbicide to reduce variation in wind, humidity, and temperature. Wind speeds were recorded at 1-s intervals during the application. Relative humidity and temperature were recorded at the beginning and end of the application. Daily weather data (wind speed, wind direction, temperature, humidity) on a 15-s interval were recorded from 1 week before application to 3 weeks after application using a weather station placed between the two fields.

Prior to application, transects were laid out in each of the eight cardinal directions extending to the edge of the field. Plots were established every 3 m from 3 to 12 m from the sprayed area, every 6 m from 12 to 36 m, every 9 m from 36 to 72 m, and every 12 m beyond 72 m until the edge of the field was reached. Two subplots consisting of four to five soybean plants per subplot were marked at each distance. The subplots consisted of soybean plants that were exposed to a) primary plus secondary drift or b) secondary drift only (any exposure more than 30 min after application). Immediately before application, 19-L buckets were placed over the soybean plants in subplots that were exposed only to secondary drift. Buckets were removed from these plants 30 minutes after completing the spray application (secondary drift only). The primary plus secondary drift subplot was never covered.

Additionally, metal rebar stands were erected with a 20 by 20 cm plywood platform affixed to the rebar at the height of the soybean canopy just before spraying. These stands were placed within the treated area and at each plot in 2015. In 2016, stands were again placed in the treated area but only in plots up to 30 m from the application. Four petri dishes (63 cm^2 in size) were placed on separate stands within the treated area to catch a full rate of dicamba. Mylar cards were placed on the stands outside of the treated area to catch primary drift. In 2015, 100 cm^2 mylar cards were placed on stands at 3, 6, 9, and 12 m from the application. Mylar cards 400 cm^2 in size were used at plots starting at 18 m to the field border. In 2016, 400 cm^2 mylar cards were used from 3 to 30 m. In order to quantify primary drift, rhodamine dye (Sigma-Aldrich Company, St. Louis, MO) was placed in each spray tank at 1 g L^{-1}. Mylar cards have been previously used as a means of catching herbicide drift (Salyani and Cromwell 1992; Yates et al. 1978). Petri dishes and mylar cards were removed from the field 30 min after application and placed in plastic bags indicating their location and then in a dark cooler to prevent photodegradation of the dye. Petri dishes and mylar cards were taken to the University of Nebraska Pesticide Application Laboratory in North Platte, NE, to quantify the amount of dye present on each surface using fluorimetry. A Turner Designs Trilogy 7200-000 (San Jose, CA) with green module and RTW/PE filter was used to analyze the samples. Samples were prepared by adding either 40 ml (Petri dishes and 100 cm^2 mylar cards) or 60 ml (400 cm^2 mylar cards) of distilled water and agitating to dissolve the rhodamine dye before extracting with a pipette and placing into 10- by 10-mm plastic cuvettes, which were placed in the fluorimeter for reading. Readings were given in relative fluorescence units (RFU) and later converted to ppm of rhodamine dye with use of a calibration curve. From ppm of rhodamine dye, concentrations could then be converted to amount of solution reaching each card, allowing calculation of the dicamba dose reaching each distance via primary movement.

Injury to soybean within each subplot (primary plus secondary, secondary) was rated at 7, 14, and 21 days after application (DAA). Injury was rated on a 0 to 100% scale with 100% being plant death. There was no attempt to solely quantify primary drift because this would have required plants be covered for several days with buckets as DGA dicamba is known to volatilize throughout this period (Anonymous 2017). Injury to soybean outside of the treated area was primarily in the form of leaf cupping, but also included leaf crinkling, epinasty, and terminal death (Andersen et al. 2004; Sciumbato et al. 2004). Two soybean plants exposed to primary plus secondary drift were harvested at 7 DAA in 2015 and four plants in 2016 directly adjacent to all distances that were rated for injury. Samples were transported on dry ice to the Arkansas State Plant Board in Little Rock, AR, and analyzed for dicamba remaining in the tissue. The method of dicamba extraction and quantification was GC/MS, similar to that reported previously (Andersen et al. 2004). The limit of detection was 1 ppb.

Analysis of Droplet Spectrum. BAPMA and DGA dicamba spray solutions similar those used in the field study were analyzed with a Sympatec Helos Vario KR particle size analyzer (Sympatec GmbH, Pulverhaus, Germany) with R7 lens installed in a low speed wind tunnel at 24 km h^{-1}. Droplets were detectable from 18 to 3500 microns. This equipment uses laser diffraction to determine particle size distribution, and the width of the spray pattern was analyzed by moving the nozzle across the laser with a linear actuator. A single TeeJet 11003 TTI nozzle was used with a pressure of 275 kPa.

Dose Response Experiment. Credenz 4950 was also planted on the same day as the large field experiment in a smaller field located approximately 1 km away for use as a DGA and BAPMA dicamba rate response experiment. Applications were made on the same day as the large field experiment. Row spacing, irrigation, and weed control measures were also the same as in the large field experiment. Ten dicamba doses (56, 17.5, 5.6, 1.75, 0.56, 0.175, 0.056, 0.0175, 0.0056, and 0.00175 g ae ha^{-1}) for each formulation were applied to the center two rows of each four-row plot using a CO_2-pressurized backpack sprayer with a 1.5-m spray boom equipped with four 110015 AIXR nozzles (TeeJet Technologies, Springfield, IL, 62703) with an output of 143 L ha^{-1} at 275 kPa. Treatments were arranged in a randomized complete block design and included four replications.

Injury ratings were taken 7, 14, and 21 DAA. Data were subjected to a two-way ANOVA to test for effects of rate, formulation, and the interaction between rate and formulation as related to injury at 21 DAA. Injury data were also subjected to regression analysis using Sigma Plot (Systat Software Inc., San Jose, CA) to determine goodness of fit based on r^2, AIC (Akaike information criterion), and BIC (Bayesian information criterion) values and significance of the regression ($\alpha < 0.05$). For each year, a model describing ln dose (g ae ha^{-1}) as a function of injury (%) at 21 DAA was produced. Models could then be applied to their respective years within the large drift experiment where observed injury could be paired with an estimated rate of dicamba in g ae ha^{-1} at that particular location within the field similar to that done previously (Egan and Mortensen 2012).

Similar to the large drift trial, whole plant tissue samples were collected 7 DAA (DGA salt only) and analyzed for the presence of dicamba. Plant heights were also collected 21 DAA and subjected non-linear regression analysis in Sigma Plot (Systat Software Inc., San Jose, CA). Various exponential models were tested and goodness of fit was decided based on r^2, AIC, and BIC values. Measures of AIC and BIC were used to compare across models with the lowest values indicating the best fit. Regression figures for the effect of dicamba dose on soybean height were produced using JMP Pro 12 (SAS Institute, Cary, NC).

Results and Discussion

Large Drift Experiment. Most volatility of dicamba occurs in the first 24 hours after application; however, volatility can occur for at least 3 days after application (Anonymous 2017; Behrens and Lueschen 1979; Mueller et al. 2013). No attempt was made to quantify primary drift injury because buckets placed on plants for this period would likely result in significant plant injury because of the hot conditions experienced the day of application. Therefore, only injury seen from secondary and primary plus secondary off-target movement of dicamba to soybean is discussed.

Ambient air temperature was 38 C in 2015 and 30 C in 2016 at the time of application whereas relative humidity was 44% in 2015 and 77% in 2016 (Table 1). Environmental conditions during application were a good representation of those likely for a POST herbicide applied to late-planted or double-crop soybean. Wind speed ranged from 4 to 12 km h^{-1} in 2015 and 10 to 16 km h^{-1} in 2016, conditions suitable for spraying based on the label for the BAPMA salt of dicamba in 2017 (Anonymous 2016b). Winds were primarily in a north/northeastern direction during and for 48 h after application both years (Figure 1); therefore, soybean injury was mainly confined to the north, northeast, and east transects (Tables 2 through 14); only transects having injury are presented in tables.

Injury resulting from primary plus secondary drift generally occurred along transects at further distances following application of the DGA than the BAPMA salt of dicamba in 2015 (Tables 2 through 6). In the 2015 experiment, the maximum distance to soybean injury via primary plus secondary drift was 45 m for DGA and 30 m for BAPMA, as indicated by an average 1% soybean injury in the DGA experiment at 21 DAA. Yet, this slight malformation may not be noticeable to the average grower. The distance to 5% injury was 30 m for DGA and 24 m for BAPMA.

Primary plus secondary drift of dicamba was detected at much greater distances in 2016, likely caused by wind speed being greater, as wind velocity is reported to have a linear relationship with drift of herbicide spray (Maybank et al. 1978) (Tables 7 through 14). Soybean injury via primary plus secondary drift occurred up to the field edge (over 180 m) with the DGA salt and extended to 108 m with the BAPMA salt. The maximum distance to 5% soybean injury of the DGA salt (120 m) was over twice as far as the BAPMA salt (54 m).

The droplet spectrum of a given nozzle may be dependent upon the mixture being applied (Meyer et al. 2015). Meyer et al. (2015) documented volume median diameter (VMD; the point at which 50% of the spray volume is below the given size) for a 1X rate of glufosinate (594 g ai ha^{-1}) to be 617 µm when applied through TTI 11006 nozzles at a pressure of 275 kPa. Using the same nozzle and pressure, VMD for a 1X rate of BAPMA dicamba (560 g ae ha^{-1}) was 756 µm. However, our results document the difference in VMD to be just 13 microns between DGA (757 µm) and BAPMA dicamba (744 µm). In addition, the percentage of fines (droplets < 210 µm) was equivalent for the two formulations (1.57% of total spray volume). Therefore, similar distance primary drift would be expected.

An attempt to measure primary drift using mylar cards resulted in only two positive readings in 2015 and nine positive readings in 2016. Use of mylar cards in combination with fluorimetry does not appear accurate enough to quantify the extremely low rates of primary dicamba drift capable of causing injury to soybean. Conversely, dicamba drift research in a wind tunnel using a 1,3,6,8-pyrenetetrasulfonic acid tetra-sodium salt (PTSA) fluorescent tracer in conjunction with 1.2 by 0.5-m polyethylene rugs to absorb droplets has provided better results (Alves et al. 2017a; 2017b). The confined system in combination with a larger surface area to collect droplets may be why the wind tunnel evaluations were more successful than field estimates of drift. Additionally, it may be possible that rhodamine dye was lost during the 30-min period following application. As shown in other research, rhodamine dye is sensitive to photo-degradation (Wu et al. 1998).

Weather conditions can drastically affect secondary off-target movement of dicamba with air temperature being positively correlated and humidity being negatively correlated with volatility (Behrens and Lueschen 1979; Mueller et al. 2013). Higher temperature accompanied with low humidity in 2015 would likely lead to greater volatile loss than the moderate temperature and humidity level that occurred at application in 2016. However, secondary movement was less in 2015 when compared to 2016. A 7-mm rain event 8 hours following application in 2015 likely caused some dicamba to be washed from soybean leaves and incorporated into soil, greatly reducing subsequent volatility (Behrens and Leuschen 1979). As a result, secondary injury was observed only out to 24 m with the DGA salt and 12 m with the BAPMA salt in 2015. The 2016 experiment led to secondary injury out to 180 m with the DGA salt and 108 m with the BAPMA salt. No precipitation occurred for 3 days following the 2016 experiment.

Rate Response Experiment. A two-parameter exponential model was fit to the soybean height data both years (Figures 2 and 3; Table 15). The curve for 2016 was much steeper than 2015, and the highest dicamba rates produced nearly twice the height reduction in 2016.

Soybean injury in the rate response experiment mirrored that of the large drift experiments in that malformation was much greater in 2016 than 2015 (Figures 4 and 5). Again, it is thought that either environmental conditions around the time of application or the unexpected rainfall after application caused such differences. Soybean injury reached a maximum at 21 DAA; therefore, this measure was used in all evaluations.

There was no significant difference between formulations and no interaction between formulation and rate in either year; therefore, data were pooled over formulations each year. Previous research has established similar findings regarding DMA and DGA salts of dicamba

(Egan and Mortensen 2012). In both years, a quadratic model described the relationship between soybean injury and rate applied. Models for each respective year were used to estimate an approximate dose of dicamba received in plots of the large drift experiment. The results are presented in Tables 2 through 14.

The amount of dicamba estimated to reach subplots as calculated by injury from the rate response experiment was numerically greater for DGA than for BAPMA both years. This may be due to the volatile component being less for BAPMA dicamba or the heavier weight resulting in a greater settling velocity. In 2016, estimations of injury were also greater, and damage extended further from the area applied for both herbicides.

Analytical Detection of Dicamba. Overall, results from analytical detection of dicamba in soybean tissue were variable (Table 16). Dicamba was recovered in greater quantities in 2016 than 2015. In 2015, only seven plots from the rate titration experiment tested positive for dicamba, and no plants treated with dicamba lower than 5.6 g ha^{-1} tested positive for dicamba. In 2016, dicamba was detected at rates as low as 1.75 g ha^{-1}. It could be that the 7 mm rainfall event approximately 6 hours after application in 2015 affected dicamba adsorption. Information in the literature is limited on absorption of dicamba in soybean; however, some research exists in weed species. One such article documented that ^{14}C uptake of dicamba only reached 47 and 33% of that applied at 7 days after application to resistant and susceptible kochia (*Kochia scoparia* L. Schrad.), respectively (Cranston et al. 2001). At one day after application, both were reported to adsorb less than 15% of the ^{14}C dicamba applied. Assuming that adsorption of dicamba is somewhat similar between kochia and soybean, it is likely that some dicamba was washed from leaf surfaces and allowed to either volatilize or move to the soil and result in less total dicamba plant adsorption in 2015.

Similar injury ratings were documented between the large drift and rate response experiments. However, dicamba was recovered at greater concentrations in the rate response experiment (Figures 6 and 7). More uniform coverage and a higher spray volume in the rate response experiment could have led to greater uptake of dicamba. More volatilization likely occurred in the large drift experiments than in rate titration experiments because the amount of dicamba applied was greater. Additionally, it is possible that dicamba uptake from primary deposition is not equal to that of gaseous entry of the herbicide. However, an assumption made in other research was that injury to soybean from low-rate direct applications is comparable to injury from volatilization (Egan and Mortensen 2012). No literature is available comparing the two forms of uptake at present. Gas exchange allowing uptake of volatile dicamba may be occurring at a higher rate than adsorption of dicamba salt through cuticular waxes and membranes, which could further complicate research pertaining to off-target movement of dicamba.

Even in plots having 25 to 40% injury, the presence of dicamba could not always be detected in the soybean tissue, meaning that individuals collecting tissue following observed injury caused by dicamba may obtain a false negative (plants showing symptoms with no dicamba analytically detected) from an analytical report (Figures 6 and 7). The variability in data along with false negatives seem to indicate that visible injury ratings may detect dicamba more accurately and efficiently than the analytical methods employed in this experiment. Previous research by Andersen et al. (2004) also attempted to recover dicamba residue from soybean foliage. Their research proposes that dicamba is either translocated to roots or is metabolized by aboveground meristematic tissue as the ability to recover dicamba from foliage diminished rapidly over time. Other research documented 10 and 64.5% of 5-OH dicamba (dicamba metabolite) was found in the treated leaf of susceptible and resistant kochia, respectively, at 7 days after application of ^{14}C dicamba (Cranston et al. 2001).

Practical Implications. Results from the rate response study indicate that soybean is equally sensitive to dicamba formulations containing the DGA or BAPMA salts when exposed to low rates at vegetative stages. In other research, Egan and Mortensen (2012) found no difference in soybean sensitivity between dicamba formulations of DGA and DMA salts. However, the distance to soybean having 5% secondary injury was reduced by half in the BAPMA large drift experiment in 2016. Hence, BAPMA dicamba may be a more responsible choice for application in DR soybean and cotton. Yet it must be noted that in 2016 BAPMA dicamba moved 108 m (1% injury) via secondary drift and was documented to cause 5% injury at 63 m from only a 1,444 m^2 (0.14 ha) application area. With use of BAPMA dicamba in DR-crops, application areas will increase. It is likely that secondary movement of dicamba will also increase at a proportional rate, causing injury to nearby non-DR soybean. Our research could also be a best-case scenario, in that previous research has shown volatile loss of dicamba to be increased after it contacts soybean foliage than when it is deposited on a silt loam soil (Behrens and Lueschen 1979). Hence, if a dicamba application is delayed to a late vegetative stage when more foliage exists, volatile loss and subsequent secondary drift could be magnified.

A larger area was injured in 2016 than in 2015. The reduced injury in 2015 could be due to the rain event shortly after application. The rainfall likely did not allow time for adequate adsorption of dicamba and reduced subsequent volatilization, essentially resulting in plants being exposed to lower rates of dicamba in 2015. Even more compounding is the timing of POST herbicide application in these trials. In 2015, POST applications of glufosinate were made one week after initiation of the drift event. However, in 2016 a POST glufosinate application was made 3 days prior to the drift event. Previous research has documented that some herbicides may increase soybean injury incurred from dicamba when applied simultaneously (Kelley et al. 2005). Additionally, unabsorbed glufosinate on the leaf surface could potentially cause enhanced dicamba volatilization as seen in other research where dicamba volatility increased when mixed with dicamba (Norsworthy 2017, unpublished data).

Based on the dicamba residue results, it does not appear likely that the analytical methods employed are sufficient for detecting dicamba in soybean, even when tissue samples are collected as early as 7 days after a drift event. The fact that dicamba cannot be easily detected using analytical techniques may be extremely important when trying to determine the actual auxin herbicide responsible for injury to soybean, especially when multiple auxin herbicides are used for preplant and in-crop applications in an array of crops.

Primary drift may be adequately mitigated by use of downwind buffers and application practices, but secondary drift is not easily resolved. With primary drift, wind direction during application provides insight into risk for injury to susceptible crops in the downwind direction; however, injury from secondary movement resulting from changes in wind direction following application poses a risk that is difficult to account for during the application. Ultimately, a single-direction buffer may be adequate for primary drift; however, multi-directional buffers are necessary to protect non-DR soybean and other sensitive vegetation from secondary drift.

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Table 1. Weather conditions during and after application of DGA and BAPMA dicamba in 2015 and 2016 at Keiser, AR.[a,b]

Time period	Rainfall		Min. /max. air temperature		Min. /max. relative humidity	
	2015	2016	2015	2016	2015	2016
	--mm--		------C------		------%------	
During application	--	--	38	31	45	77
Day of application	7	0	23/38	27/31	44/87	65/83
One day after application	3	0	23/30	24/32	62/91	58/89
Two days after application	22	0	22/29	24/33	65/94	53/91
Three days after application	2	0	23/30	22/30	60/93	62/91

[a]Abbreviations: DGA, diglycolamine salt of dicamba; BAPMA, N,N-Bis-(aminopropyl) methylamine salt of dicamba; min, minimum; max, maximum

[b] Average/maximum wind speed during application was 8/12 and 12/16 km h^{-1} in 2015 and 2016, respectively. During application wind was in the N direction in 2015 and in the NNE direction in 2016. See Figure 1 for average wind speed and direction following application.

Table 2. Injury to soybean, estimated dose of DGA and BAPMA dicamba, DGA dicamba detected in soybean, and dicamba detected on mylar cards along the north transect in 2015 at Keiser, AR.[ab]

	DGA					BAPMA			
	Injury[c]					Injury[c]			
Distance[g]	Primary + Secondary	Secondary	Estimated dose[d]	Foliar residue[e]	Mylar residue[f]	Primary + Secondary	Secondary	Estimated dose[d]	Mylar residue[f]
m	---------%---------		g ae ha^{-1}	--------ppb--------		---------%---------		g ae ha^{-1}	ppb
3	45	20	30.994	12	0	35	15	19.248	0
6	35	15	19.248	10	0	30	10	12.287	0
9	25	5	6.816	14	0	30	7	12.287	0
12	20	5	3.286	0	0	25	5	6.816	0
18	15	0	1.376	0	0	12	0	0.764	0
24	8	0	0.322	14	0	5	0	0.159	0
30	7	0	0.255	0	0	1	0	0.057	0
36	1	0	0.057	0	0	0	0	0	0

[a]Abbreviations: DGA, diglycolamine form of dicamba; BAPMA, N,N-Bis-(aminopropyl) methylamine form of dicamba

[b]Wind direction during application ranged between NNE and NNW with an average of 8 and max of 12 km h^{-1}

[c]Plant injury rated on a 0 to 100% scale with 100% being plant death

[d]Dose estimated using equations generated from rate titration trial injury levels

[e]The limit for detecting dicamba was 1 ppb

[f]The estimated amount of dicamba collected from mylar cards placed within plots for measuring physical drift from 0 to 30 minutes after application

[g]Distances where no injury was observed are not shown

Table 3. Injury to soybean, estimated dose of DGA and BAPMA dicamba, and DGA dicamba detected in soybean along the northeast transect in 2015 at Keiser, AR.[ab]

	DGA					BAPMA			
	Injury[c]					Injury[c]			
Distance[g]	Primary + Secondary	Secondary	Estimated dose[d]	Foliar residue[e]	Mylar residue[f]	Primary + Secondary	Secondary	Estimated dose[d]	Mylar residue[f]
m	---------%---------		g ae ha^{-1}	--------ppb--------		---------%---------		g ae ha^{-1}	ppb
3	40	8	0.096	14	0	25	10	6.816	0
6	30	5	12.287	8	0	20	8	3.286	0
9	20	5	3.286	0	57	18	5	2.359	0
12	10	2	0.501	8	0	15	2	1.376	0
18	7	1	0.256	0	0	8	0	0.322	0
24	5	1	0.159	0	0	3	0	0.096	0
30	5	0	0.159	0	0	2	0	0.074	0
36	4	0	0.1238	0	0	0	0	0	0
45	1	0	0.057	0	0	0	0	0	0

[a]Abbreviations: DGA, diglycolamine form of dicamba; BAPMA, N,N-Bis-(aminopropyl) methylamine form of dicamba

[b]Wind direction during application ranged between NNE and NNW with an average of 8 and max of 12 km h^{-1}

[c]Plant injury rated on a 0 to 100% scale with 100% being plant death

[d]Dose estimated using equations generated from rate titration trial injury levels

[e]The limit for detecting dicamba was 1 ppb

[f]The estimated amount of dicamba collected from mylar cards placed within plots for measuring physical drift from 0 to 30 minutes after application

[g]Distances where no injury was observed are not shown

Table 4. Injury to soybean, estimated dose of DGA and BAPMA dicamba, and DGA dicamba detected in soybean along the east transect in 2015 at Keiser, AR.[ab]

	DGA					BAPMA			
	Injury[c]					Injury[c]			
Distance[g]	Primary + Secondary	Secondary	Estimated dose[d]	Foliar residue[e]	Mylar residue[f]	Primary + Secondary	Secondary	Estimated dose[d]	Mylar residue[f]
m	---------%---------		g ae ha^{-1}	--------ppb--------		---------%---------		g ae ha^{-1}	ppb
3	30	8	12.287	10	0	25	1	6.816	0
6	15	5	1.376	11	0	15	1	1.376	0
9	7	1	0.255	10	0	10	0	0.501	0
12	2	0	0.074	0	0	5	0	0.159	0
18	0	0	0	0	0	2	0	0.074	0

[a]Abbreviations: DGA, diglycolamine form of dicamba; BAPMA, N,N-Bis-(aminopropyl) methylamine form of dicamba

[b]Wind direction during application ranged between NNE and NNW with an average of 8 and max of 12 km h^{-1}

[c]Plant injury rated on a 0 to 100% scale with 100% being plant death

[d]Dose estimated using equations generated from rate titration trial injury levels

[e]The limit for detecting dicamba was 1 ppb

[f]The estimated amount of dicamba collected from mylar cards placed within plots for measuring physical drift from 0 to 30 minutes after application

[g]Distances where no injury was observed are not shown

Table 5. Injury to soybean, estimated dose of DGA and BAPMA dicamba, and DGA dicamba detected in soybean along the southeast transect in 2015 at Keiser, AR.[ab]

	DGA					BAPMA			
	Injury[c]					Injury[c]			
Distance[g]	Primary + Secondary	Secondary	Estimated dose[d]	Foliar residue[e]	Mylar residue[f]	Primary + Secondary	Secondary	Estimated dose[d]	Mylar residue[f]
m	---------%---------		g ae ha^{-1}	--------ppb--------		---------%---------		g ae ha^{-1}	ppb
3	0	0	0	0	0	2	0	0.074	0

[a]Abbreviations: DGA, diglycolamine form of dicamba; BAPMA, N,N-Bis-(aminopropyl) methylamine form of dicamba

[b]Wind direction during application ranged between NNE and NNW with an average of 8 and max of 12 km h^{-1}

[c]Plant injury rated on a 0 to 100% scale with 100% being plant death

[d]Dose estimated using equations generated from rate titration trial injury levels

[e]The limit for detecting dicamba was 1 ppb

[f]The estimated amount of dicamba collected from mylar cards placed within plots for measuring physical drift from 0 to 30 minutes after application

[g]Distances where no injury was observed are not shown

Table 6. Injury to soybean, estimated dose of DGA and BAPMA dicamba, and DGA dicamba detected in soybean along the south transect in 2015 at Keiser, AR.[ab]

	DGA					BAPMA			
	Injury[c]					Injury[c]			
Distance[g]	Primary + Secondary	Secondary	Estimated dose[d]	Foliar residue[e]	Mylar residue[f]	Primary + Secondary	Secondary	Estimated dose[d]	Mylar residue[f]
m	---------%---------		g ae ha^{-1}	--------ppb--------		---------%---------		g ae ha^{-1}	ppb
3	45	45	30.994	880	0	1	1	0.057	78592
6	15	0	1.376	13	0	0	0	0	0
9	0	0	0	19	0	0	0	0	0
12	0	0	0	21	0	0	0	0	0

[a]Abbreviations: DGA, diglycolamine form of dicamba; BAPMA, N,N-Bis-(aminopropyl) methylamine form of dicamba

[b]Wind direction during application ranged between NNE and NNW with an average of 8 and max of 12 km h^{-1}

[c]Plant injury rated on a 0 to 100% scale with 100% being plant death

[d]Dose estimated using equations generated from rate titration trial injury levels

[e]The limit for detecting dicamba was 1 ppb

[f]The estimated amount of dicamba collected from mylar cards placed within plots for measuring physical drift from 0 to 30 minutes after application

[g]Distances where no injury was observed are not shown

Table 7. Injury to soybean, estimated dose of DGA and BAPMA dicamba, and DGA dicamba detected in soybean along the north transect in 2016 at Keiser, AR.[ab]

	DGA					BAPMA			
	Injury[c]					Injury[c]			
Distance[g]	Primary + Secondary	Secondary	Estimated dose[d]	Foliar residue[e]	Mylar residue[f]	Primary + Secondary	Secondary	Estimated dose[d]	Mylar residue[f]
m	---------%---------		g ae ha^{-1}	--------ppb--------		---------%---------		g ae ha^{-1}	ppb
3	55	40	17.292	131	1,658	55	50	17.292	2,739
6	60	45	24.818	44	0	60	50	24.818	0
9	45	40	6.995	0	0	65	40	33.521	0
12	50	40	11.338	17	0	48	40	9.415	0
18	45	35	6.995	0	0	40	32	4.062	0
24	35	30	2.22	0	0	40	40	4.062	0
30	25	15	0.552	0	0	28	15	0.86	0
36	20	15	0.252	0	2,930	20	10	0.252	0
45	20	15	0.252	0	-	15	8	0.108	-
54	15	10	0.108	0	-	10	5	0.043	-
63	10	5	0.043	0	-	5	3	0.017	-
72	8	7	0.03	0	-	5	2	0.017	-
84	7	5	0.024	0	-	5	2	0.017	-
96	7	5	0.024	0	-	5	1	0.017	-
108	8	4	0.03	0	-	3	1	0.011	-
120	5	5	0.017	0	-	1	0	0.005	-
132	5	3	0.017	0	-	0	0	0	-
144	7	3	0.024	0	-	0	0	0	-
156	7	3	0.024	0	-	0	0	0	-

Table 7 continued

	DGA					BAPMA			
	Injury[c]		Estimated	Foliar	Mylar	Injury[c]		Estimated	Mylar
Distance[g]	Primary + Secondary	Secondary	dose[d]	residue[e]	residue[f]	Primary + Secondary	Secondary	dose[d]	residue[f]
168	5	3	0.017	0	-	0	0	0	-
180	2	1	0.008	0	-	0	0	0	-

[a]Abbreviations: DGA, diglycolamine form of dicamba; BAPMA, N,N-Bis-(aminopropyl) methylamine form of dicamba

[b]Wind direction during application ranged between NNE and NNW with an average of 8 and max of 12 km h^{-1}

[c]Plant injury rated on a 0 to 100% scale with 100% being plant death

[d]Dose estimated using equations generated from rate titration trial injury levels

[e]The limit for detecting dicamba was 1 ppb

[f]The estimated amount of dicamba collected from mylar cards placed within plots for measuring physical drift from 0 to 30 minutes after application

[g]Distances where no injury was observed are not shown

Table 8. Injury to soybean, estimated dose of DGA and BAPMA dicamba, and DGA dicamba detected in soybean along the northeast transect in 2016 at Keiser, AR.[ab]

	DGA					BAPMA			
	Injury[c]					Injury[c]			
Distance[g]	Primary + Secondary	Secondary	Estimated dose[d]	Foliar residue[e]	Mylar residue[f]	Primary + Secondary	Secondary	Estimated dose[d]	Mylar residue[f]
m	---------%---------		g ae ha^{-1}	--------ppb--------		---------%---------		g ae ha^{-1}	ppb
3	50	40	11.338	167	0	55	45	17.292	1,130
6	50	35	11.338	148	0	50	40	11.338	0
9	45	35	6.995	0	0	45	40	6.995	0
12	45	35	6.995	0	0	45	30	6.995	0
18	40	30	4.062	0	0	38	30	3.213	0
24	45	30	6.995	0	0	35	25	2.22	0
30	40	30	4.062	0	0	30	20	1.141	0
36	45	28	6.995	0	0	20	10	0.252	0
45	35	25	2.22	0	-	15	7	0.108	-
54	45	25	6.995	0	-	10	5	0.043	-
63	20	15	0.252	0	-	7	5	0.024	-
72	10	7	0.043	0	-	5	2	0.017	-
84	5	2	0.017	0	-	5	1	0.017	-
96	5	3	0.017	0	-	0	0	0	-
108	3	1	0.011	0	-	0	0	0	-

[a]Abbreviations: DGA, diglycolamine form of dicamba; BAPMA, N,N-Bis-(aminopropyl) methylamine form of dicamba

[b]Wind direction during application ranged between NNE and NNW with an average of 8 and max of 12 km h^{-1}

[c]Plant injury rated on a 0 to 100% scale with 100% being plant death

[d]Dose estimated using equations generated from rate titration trial injury levels

Table 8 continued

[e]The limit for detecting dicamba was 1 ppb
[f]The estimated amount of dicamba collected from mylar cards placed within plots for measuring physical drift from 0 to 30 minutes after application
[g]Distances where no injury was observed are not shown

Table 9. Injury to soybean, estimated dose of DGA and BAPMA dicamba, and DGA dicamba detected in soybean along the east transect in 2016 at Keiser, AR.[ab]

	DGA					BAPMA			
	Injury[c]					Injury[c]			
Distance[g]	Primary + Secondary	Secondary	Estimated dose[d]	Foliar residue[e]	Mylar residue[f]	Primary + Secondary	Secondary	Estimated dose[d]	Mylar residue[f]
m	---------%---------		g ae ha^{-1}	--------ppb--------		---------%---------		g ae ha^{-1}	ppb
3	45	35	6.995	167	1,280	45	38	6.995	0
6	50	38	11.338	29	0	55	38	17.292	0
9	45	38	6.995	0	0	50	38	11.338	0
12	28	20	0.86	0	0	35	25	2.22	0
18	25	18	0.552	0	0	25	15	0.552	0
24	15	10	0.108	0	0	25	15	0.552	0
30	20	5	0.252	0	0	25	10	0.552	0
36	10	5	0.043	0	0	15	8	0.108	0
45	8	4	0.03	0	-	5	1	0.017	-
54	10	5	0.043	0	-	3	1	0.011	-
63	8	5	0.03	0	-	0	0	0	-
72	5	2	0.017	0	-	0	0	0	-

[a]Abbreviations: DGA, diglycolamine form of dicamba; BAPMA, N,N-Bis-(aminopropyl) methylamine form of dicamba

[b]Wind direction during application ranged between NNE and NNW with an average of 8 and max of 12 km h^{-1}

[c]Plant injury rated on a 0 to 100% scale with 100% being plant death

[d]Dose estimated using equations generated from rate titration trial injury levels

[e]The limit for detecting dicamba was 1 ppb

[f]The estimated amount of dicamba collected from mylar cards placed within plots for measuring physical drift from 0 to 30 minutes after application

[g]Distances where no injury was observed are not shown

Table 10. Injury to soybean, estimated dose of DGA and BAPMA dicamba, and DGA dicamba detected in soybean along the southeast transect in 2016 at Keiser, AR.[ab]

	DGA					BAPMA			
	Injury[c]					Injury[c]			
Distance[g]	Primary + Secondary	Secondary	Estimated dose[d]	Foliar residue[e]	Mylar residue[f]	Primary + Secondary	Secondary	Estimated dose[d]	Mylar residue[f]
m	---------%---------		g ae ha^{-1}	--------ppb--------		---------%---------		g ae ha^{-1}	ppb
3	5	3	0.017	0	0	20	8	0.252	0
6	7	3	0.024	0	0	15	8	0.108	0
9	8	2	0.03	0	0	15	7	0.108	0
12	7	2	0.024	0	0	10	5	0.043	0
18	10	5	0.043	0	0	10	4	0.043	0
24	5	2	0.017	0	0	8	3	0.03	0
30	5	2	0.017	0	0	10	3	0.043	0
36	7	2	0.024	0	0	8	3	0.03	0
45	5	2	0.017	0	-	5	2	0.017	-
54	5	1	0.017	0	-	3	0	0.011	-
63	7	3	0.024	0	-	3	1	0.011	-
72	2	2	0.008	0	-	2	0	0.008	-
84	2	0	0.008	0	-	0	0	0	-

[a]Abbreviations: DGA, diglycolamine form of dicamba; BAPMA, N,N-Bis-(aminopropyl) methylamine form of dicamba

[b]Wind direction during application ranged between NNE and NNW with an average of 8 and max of 12 km h^{-1}

[c]Plant injury rated on a 0 to 100% scale with 100% being plant death

[d]Dose estimated using equations generated from rate titration trial injury levels

[e]The limit for detecting dicamba was 1 ppb

[f]The estimated amount of dicamba collected from mylar cards placed within plots for measuring physical drift from 0 to 30 minutes after application

[g]Distances where no injury was observed are not shown

Table 11. Injury to soybean, estimated dose of DGA and BAPMA dicamba, and DGA dicamba detected in soybean along the south transect in 2016 at Keiser, AR.[ab]

	DGA					BAPMA			
	Injury[c]					Injury[c]			
Distance[g]	Primary + Secondary	Secondary	Estimated dose[d]	Foliar residue[e]	Mylar residue[f]	Primary + Secondary	Secondary	Estimated dose[d]	Mylar residue[f]
m	---------%---------		g ae ha^{-1}	--------ppb--------		---------%---------		g ae ha^{-1}	ppb
3	20	15	0.252	0	0	20	10	0.252	0
6	10	10	0.043	0	0	10	5	0.043	0
9	7	2	0.024	0	0	8	3	0.03	0
12	5	2	0.017	0	4,095	3	1	0.011	159,180
18	7	4	0.024	0	0	2	0	0.008	0
24	5	1	0.017	0	0	5	0	0.017	0
30	2	1	0.008	0	0	2	0	0.008	0
36	2	2	0.008	0	0	2	1	0.008	0

[a]Abbreviations: DGA, diglycolamine form of dicamba; BAPMA, N,N-Bis-(aminopropyl) methylamine form of dicamba

[b]Wind direction during application ranged between NNE and NNW with an average of 8 and max of 12 km h^{-1}

[c]Plant injury rated on a 0 to 100% scale with 100% being plant death

[d]Dose estimated using equations generated from rate titration trial injury levels

[e]The limit for detecting dicamba was 1 ppb

[f]The estimated amount of dicamba collected from mylar cards placed within plots for measuring physical drift from 0 to 30 minutes after application

[g]Distances where no injury was observed are not shown

Table 12. Injury to soybean, estimated dose of DGA and BAPMA dicamba, and DGA dicamba detected in soybean along the southwest transect in 2016 at Keiser, AR.[ab]

	DGA					BAPMA			
	Injury[c]					Injury[c]			
Distance[g]	Primary + Secondary	Secondary	Estimated dose[d]	Foliar residue[e]	Mylar residue[f]	Primary + Secondary	Secondary	Estimated dose[d]	Mylar residue[f]
m	---------%---------		g ae ha^{-1}	--------ppb--------		---------%---------		g ae ha^{-1}	ppb
3	3	2	0.011	0	940	3	2	0.011	0
6	3	3	0.011	0	0	2	1	0.008	0
9	3	2	0.011	0	3,337	2	1	0.008	0
12	1	1	0.005	0	0	2	0	0.008	0
18	0	0	0	0	0	1	0	0.005	0

[a]Abbreviations: DGA, diglycolamine form of dicamba; BAPMA, N,N-Bis-(aminopropyl) methylamine form of dicamba

[b]Wind direction during application ranged between NNE and NNW with an average of 8 and max of 12 km h^{-1}

[c]Plant injury rated on a 0 to 100% scale with 100% being plant death

[d]Dose estimated using equations generated from rate titration trial injury levels

[e]The limit for detecting dicamba was 1 ppb

[f]The estimated amount of dicamba collected from mylar cards placed within plots for measuring physical drift from 0 to 30 minutes after application

[g]Distances where no injury was observed are not shown

Table 13. Injury to soybean, estimated dose of DGA and BAPMA dicamba, and DGA dicamba detected in soybean along the west transect in 2016 at Keiser, AR.[ab]

	DGA					BAPMA			
	Injury[c]					Injury[c]			
Distance[g]	Primary + Secondary	Secondary	Estimated dose[d]	Foliar residue[e]	Mylar residue[f]	Primary + Secondary	Secondary	Estimated dose[d]	Mylar residue[f]
m	---------%---------		g ae ha^{-1}	--------ppb--------		---------%---------		g ae ha^{-1}	ppb
3	10	10	0.043	0	0	15	7	0.108	0
6	7	5	0.024	0	0	10	7	0.043	0
9	5	5	0.017	0	0	5	2	0.017	0
12	3	3	0.011	0	0	5	2	0.017	0
18	0	0	0	0	0	2	1	0.008	0
24	0	0	0	0	0	3	1	0.011	0
30	0	0	0	0	0	2	0	0.008	0

[a]Abbreviations: DGA, diglycolamine form of dicamba; BAPMA, N,N-Bis-(aminopropyl) methylamine form of dicamba

[b]Wind direction during application ranged between NNE and NNW with an average of 8 and max of 12 km h^{-1}

[c]Plant injury rated on a 0 to 100% scale with 100% being plant death

[d]Dose estimated using equations generated from rate titration trial injury levels

[e]The limit for detecting dicamba was 1 ppb

[f]The estimated amount of dicamba collected from mylar cards placed within plots for measuring physical drift from 0 to 30 minutes after application

[g]Distances where no injury was observed are not shown

Table 14. Injury to soybean, estimated dose of DGA and BAPMA dicamba, and DGA dicamba detected in soybean along the northwest transect in 2016 at Keiser, AR.[ab]

	DGA					BAPMA			
	Injury[c]					Injury[c]			
Distance[g]	Primary + Secondary	Secondary	Estimated dose[d]	Foliar residue[e]	Mylar residue[f]	Primary + Secondary	Secondary	Estimated dose[d]	Mylar residue[f]
m	---------%---------		g ae ha^{-1}	--------ppb--------		---------%---------		g ae ha^{-1}	ppb
3	5	2	0.017	0	0	18	10	0.181	0
6	3	3	0.011	0	0	15	8	0.108	0
9	1	1	0.005	0	0	10	7	0.043	0
12	1	1	0.005	0	0	10	5	0.043	0
18	5	2	0.017	0	0	5	2	0.017	0
24	3	1	0.011	0	0	5	1	0.017	0
30	1	1	0.005	0	0	3	1	0.011	0
36	2	1	0.008	0	0	2	0	0.008	0
45	2	2	0.008	0	-	1	1	0.005	-
54	1	1	0.005	0	-	0	0	0	-

[a]Abbreviations: DGA, diglycolamine form of dicamba; BAPMA, N,N-Bis-(aminopropyl) methylamine form of dicamba

[b]Wind direction during application ranged between NNE and NNW with an average of 8 and max of 12 km h^{-1}

[c]Plant injury rated on a 0 to 100% scale with 100% being plant death

[d]Dose estimated using equations generated from rate titration trial injury levels

[e]The limit for detecting dicamba was 1 ppb

[f]The estimated amount of dicamba collected from mylar cards placed within plots for measuring physical drift from 0 to 30 minutes after application

[g]Distances where no injury was observed are not shown

Table 15. Nonlinear regression parameter estimates, standard error, and confidence intervals for the 2015 and 2016 relationship between soybean injury at 21 days after application and dicamba dose.[a]

Parameter	Estimate		Standard error		Confidence interval			
					2015		2016	
	2015	2016	2015	2016	Lower 95%	Upper 95%	Lower 95%	Upper 95%
Intercept (a)	-3.133	-5.220	0.197	0.239	-3.520	-2.747	-5.689	-4.750
Linear (b)	0.272	0.210	0.034	0.015	0.206	0.339	0.180	0.240
Quadratic (c)	-0.003	-0.001	0.001	0.000	-0.004	-0.001	-0.002	-0.001

[a]A three-parameter exponential model was used

Table 16. Mean, standard deviation, standard error, and 95% confidence intervals for dicamba recovered in soybean tissue at each respective rate applied in 2015 and 2016 at Keiser, AR.[a]

					95% confidence intervals			
	Mean		Standard error		Upper	Lower	Upper	Lower
Rate	2015	2016	2015	2016	2015	2016	2015	2016
g ae ha^{-1}	----------	----------	----------	----------	ppb	----------	----------	----------
0.0175	0	0	0	0	0	0	0	0
0.056	0	0	0	0	0	0	0	0
0.175	0	0	0	0	0	0	0	0
0.56	0	0	0	0	0	0	0	0
1.75	0	40	0	31	0	0	138	58
5.6	4	8	2	8	11	-3	31	16
17.5	12	250	6	137	39	-16	685	185
56	61	1,595	29	378	185	-63	2,798	392

[a]The limit for detecting dicamba was 1 ppb

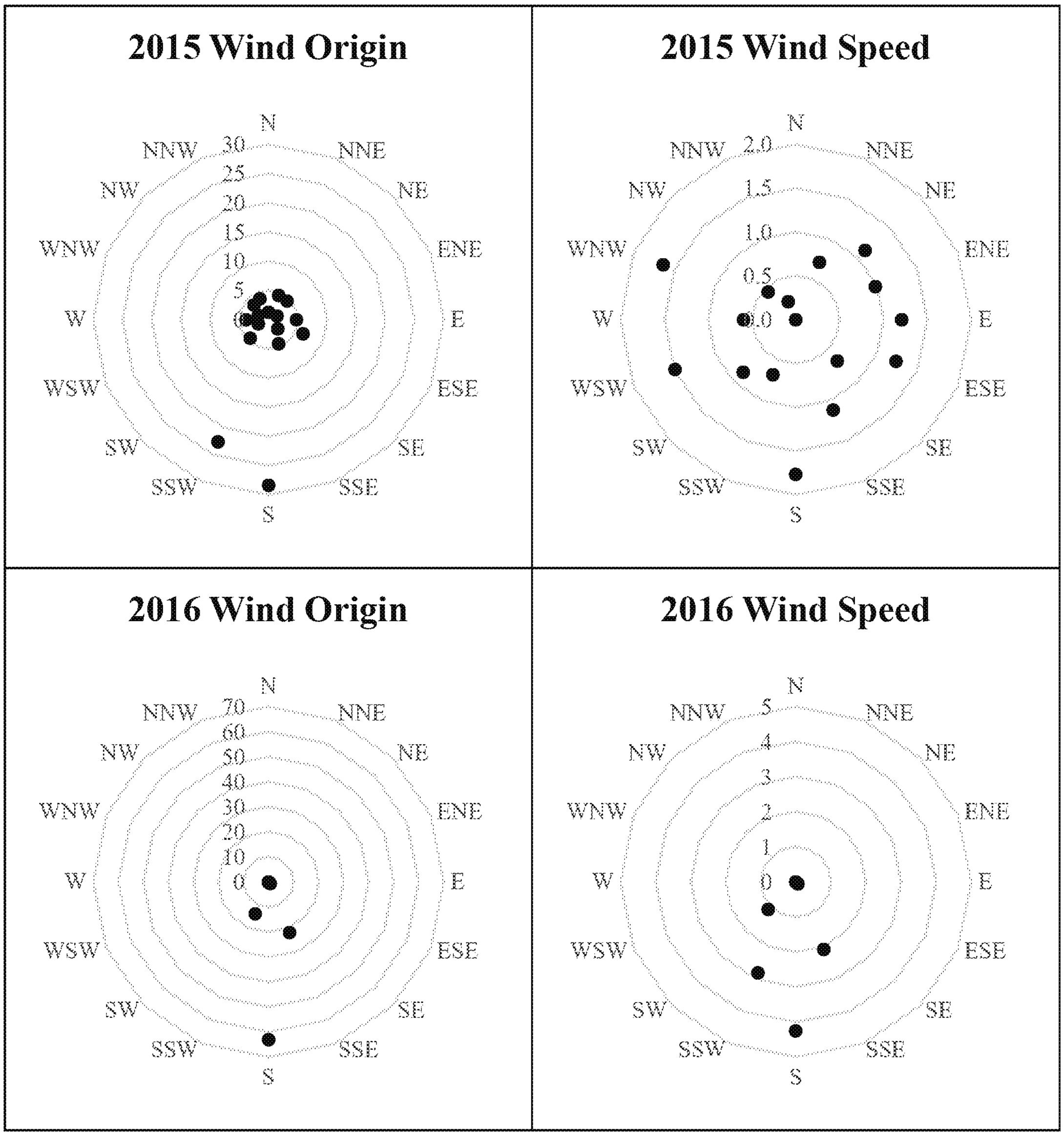

Figure 1. Web diagrams displaying wind speed and origin for 2 d after application in 2015 and 2016 at the Northeast Research and Extension Center in Keiser, AR. Wind origin is presented as percentage of all hourly measurements. Wind speed is presented as average wind speed (m s^{-1}) for each reported direction. Arrow originating from the center of each diagram indicates wind direction during application.

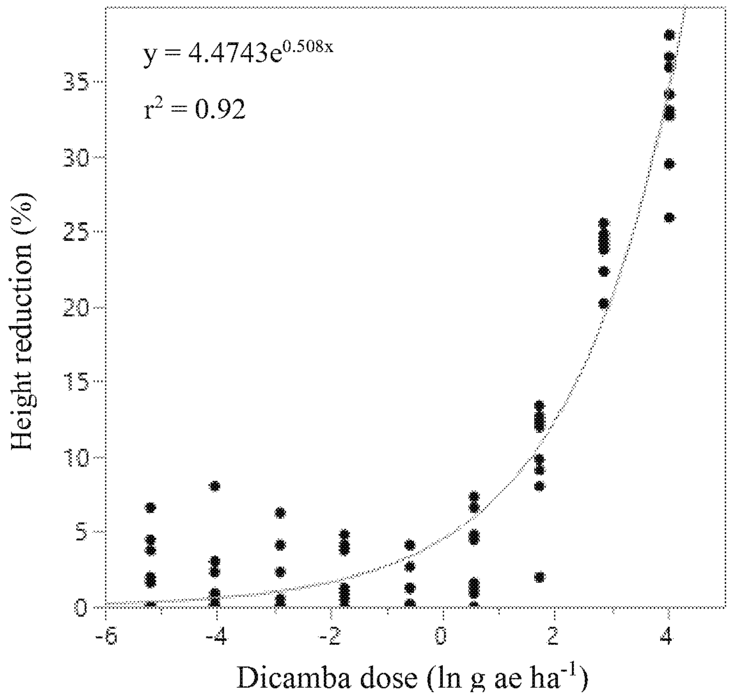

Figure 2. Two-parameter exponential growth model of the effect of dicamba dose on height reduction at 21 days after application to vegetative soybean in 2015 at Keiser, AR. Regression parameters are available in Table 16.

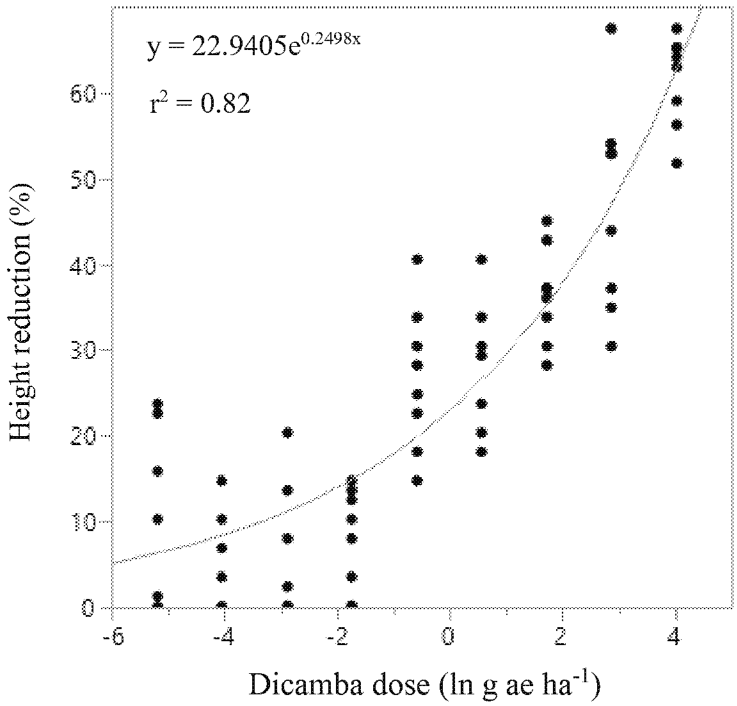

Figure 3. Two-parameter exponential growth model of the effect of dicamba dose (g ae ha-1) on height reduction at 21 days after application to vegetative soybean in 2016 at Keiser, AR. Regression parameters are available in Table 16.

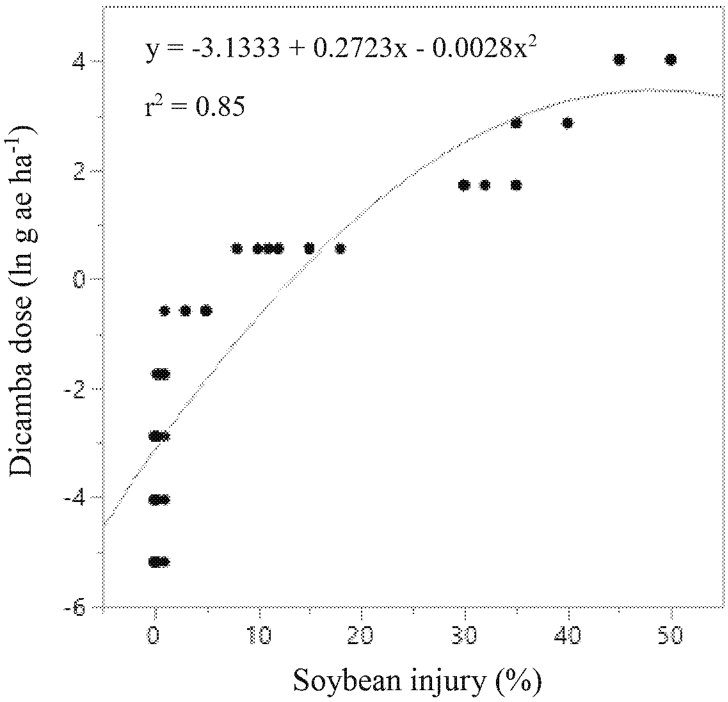

Figure 4. Quadratic model for predicting dicamba dose (g ae ha^{-1}) in the large drift experiments using soybean injury at 21 days after application in 2015 at Keiser, AR. Regression parameters are available in Table 17.

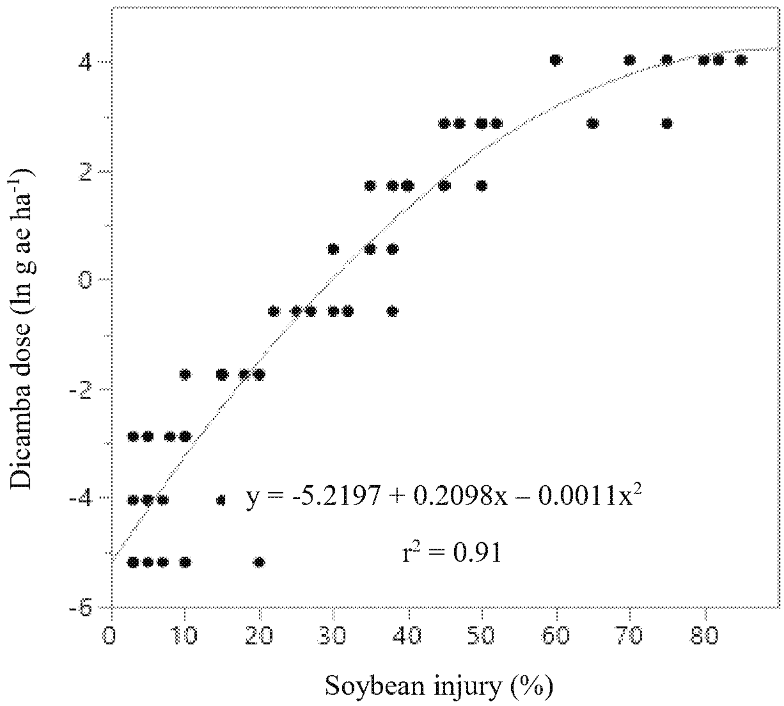

Figure 5. Quadratic model for predicting dicamba dose (g ae ha^{-1}) in the large drift experiments using soybean injury at 21 days after application in 2016 at Keiser, AR. Regression parameters are available in Table 17.

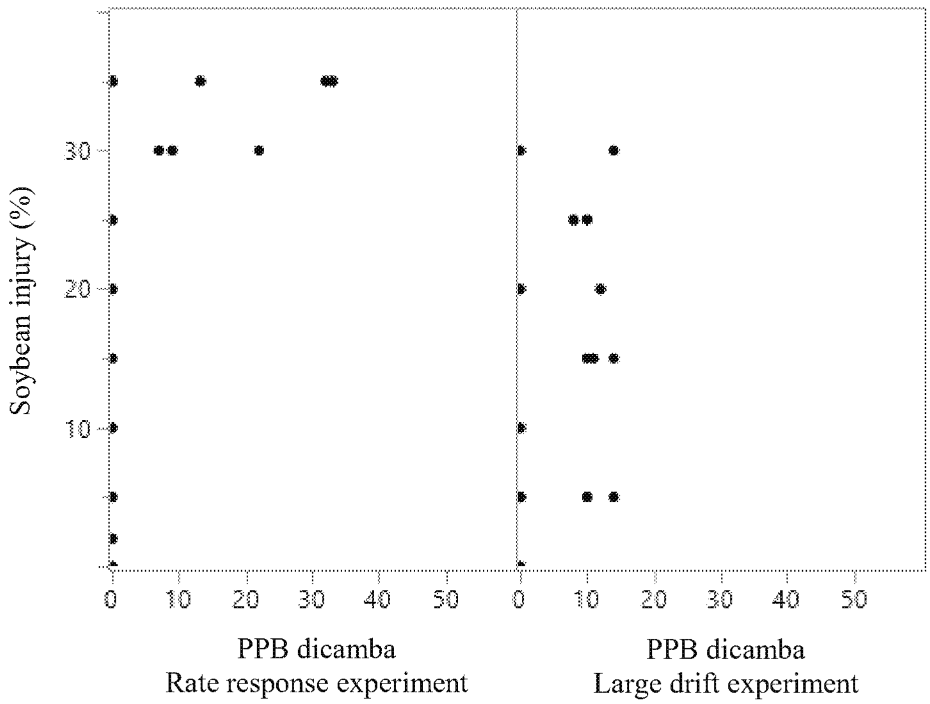

Figure 6. Scatterplot matrix of soybean injury and ppb (parts per billion) diglycolamine dicamba recovered in soybean tissue harvested at 7 days after application in 2015 at Keiser, AR. The dicamba detection limit was 1 ppb.

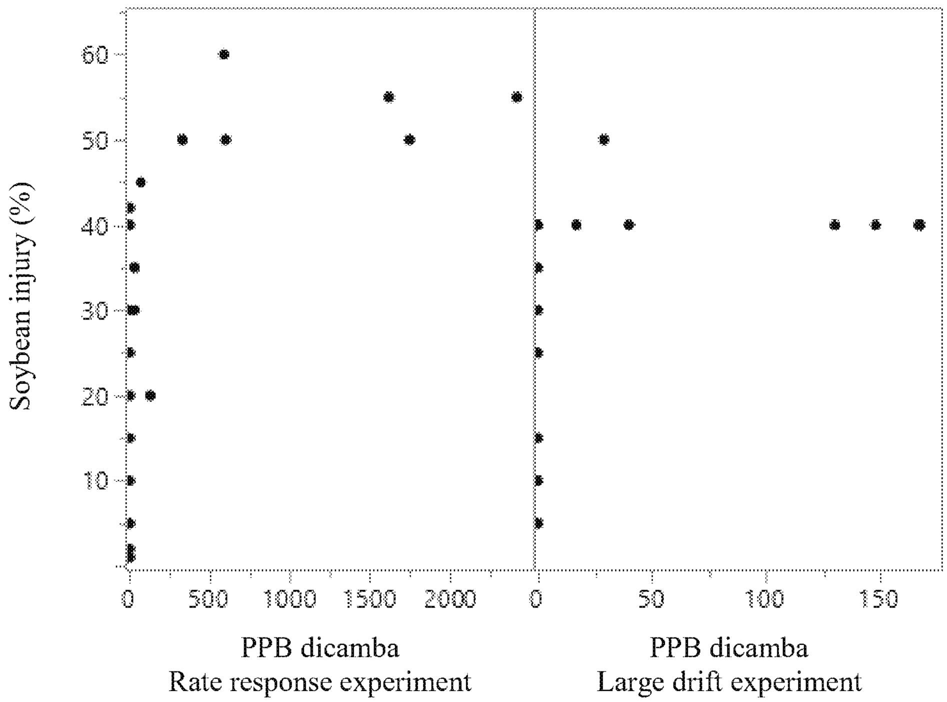

Figure 7. Scatterplot matrix of soybean injury and ppb (parts per billion) diglycolamine dicamba recovered in soybean tissue harvested at 7 days after application in 2016 at Keiser, AR. The dicamba detection limit was 1 ppb.

General Conclusions

Dicamba is highly useful in weed management programs as it will provide another postemergence site of action to control Palmer amaranth in areas where glufosinate-resistant soybean and cotton are a grower's only option. History shows that overreliance on a single herbicide may eventually lead to resistance. However, dicamba poses unique problems such as volatility and potential to damage highly susceptible crops such as non-DR soybean.

Yield reduction occurred to soybean at a distance nearly 3 times (90.4 m) that of the labeled setback (33.3 m) for endangered species at the field edge. Furthermore, soybean offspring could be at risk to negative affects when dicamba drift occurs at later reproductive stages. Soybean pod malformation when dicamba drift occurs at growth stage R5 was documented to be predictive of these negative offspring affects such as reduced emergence, reduced vigor, and injury.

Based on current label guidelines, the addition of a product to XtendiMax, FeXapan, or Engenia herbicides must be analyzed on a case-by-case basis with the sole purpose being the effect of the additive on droplet spectrum. Currently, many forms of glyphosate are labeled for mixing with these new dicamba products. Yet, in some cases, a drift reducing agent (DRA) must be used also to negate the effects that certain glyphosate formulations have on reducing droplet spectrum. However, this research demonstrates that there is another concern of such mixtures. Low-rate applications of dicamba and glyphosate were demonstrated to increase leaf malformation at R1 and increase pod malformation at R3 exposure, although negative effects on soybean offspring were not intensified by the addition of glyphosate. It is currently unclear just why increased injury occurs with low-rate exposure of the mixture, but it is hypothesized that glyphosate is aiding translocation of dicamba.

Engenia (BAPMA dicamba) was documented to have reduced secondary movement when compared to Clarity (DGA dicamba). However, secondary movement was still documented out to 108 m (1% injury) and is expected to increase in proportion to the area applied. This level of injury may be short-lived, and although not tested in these studies, it is expected that height and yield reduction will not occur at such low injury levels. However, 10 to 15% injury by secondary exposure was observed for both dicamba products at distances beyond the current buffer requirements. In addition, multiple exposures of dicamba could occur as growers will typically apply the herbicide twice with approximately two weeks between applications. Therefore, further research may be needed to evaluate the effect on height and yield of multiple dicamba exposures to non-DR soybean.

Proper stewardship of XtendiMax and Engenia herbicides will be key in their longevity. Label guidelines like those imposed on these two herbicides have not been seen previously. Proper nozzles, pressure, and boom height are vital in reducing primary drift of dicamba products. However, environmental conditions during and following application will contribute to the extent of secondary drift. While primary drift may be adequately controlled when using label guidelines, secondary movement may still occur if environmental conditions allow. In summary, these experiments demonstrate the danger that dicamba could pose on non-DR soybean as current guidelines will need to be stringently followed so that dicamba may be used to fight against problematic resistant weeds in DR soybean and cotton.